Optimization of Preparation Procedures of Phlorizin-Zn (II) Complex and Its Antioxidant Activity Study in vitro

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Abstract: Flavonoid metal complexes possess more effective antioxidant properties than flavonoids. However, the preparation of the phlorizin-Zn (II) complex and its antioxidant activity has not been reported. In this research, the preparation procedures of the phlorizin-Zn (II) complex were enhanced using single factor test and Response Surface Methodology (RSM). The optimal preparation procedures of the phlorizin-Zn (II) complex were as follows: The time of 4.1 h, the pH of 5.9, the temperature of 48.7°C and the molar ratio (phlorizin: Zn^{2+}) of 1:1. Under these conditions, the predicted preparation yield of phlorizin-Zn (II) complex was 79.052%. Additionally, the antioxidant activity of the phlorizin-Zn (II) complex was analyzed in this research. 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH), 2, 2’-Azinobis (3-Ethylbenzothiazolone-6-Sulfonic acid) (ABTS), hydroxyl free radical and Ferric ion Reducing Antioxidant Power (FRAP) experiments revealed that phlorizin-Zn (II) complex had significant DPPH, ABTS and hydroxyl free radical scavenging abilities and exhibited substantial reducing power in a dose-dependent manner. Moreover, the phlorizin-Zn (II) complex displayed stronger in vitro antioxidant capacity compared with phlorizin. The findings demonstrated that the phlorizin-Zn (II) complex had great potential in antioxidant applications in the future.

Keywords: Phlorizin-Zn (II) Complex, Preparation Procedure, Response Surface Methodology, Antioxidant Activity

Introduction

Polyphenolic secondary metabolic substances in plants are known as flavonoids (Khalid et al., 2019). There are several biological and pharmacological characteristics displayed by flavonoids, including neuroprotective, anti-inflammatory, anti-cancer, antibacterial, and antidiabetic properties (Ullah et al., 2020). Phlorizin is one of the most common flavonoids found in apples which is plentiful in both the unpeeled and peeled parts (Gosch et al., 2010). Phlorizin has several pharmacological actions, such as hypoglycemic, antioxidant, and anti-inflammatory properties (Jia et al., 2021). It was discovered that the phlorizin from lotus seed epicarp had high FRAP values and was effective in scavenging DPPH and ABTS radicals (Ma et al., 2019). Previous research showed that phlorizin increased the activity of antioxidant enzymes in Drosophila and prolong the life span, improve the survival ability, and reduce the mortality of Drosophila treated with paraquat and H_{2}O_{2} (Wang et al., 2019). Therefore, phlorizin shows great potential in antioxidant activity.

After the formation of complexes between flavonoids and metal ions, their antibacterial, anti-inflammatory, anticancer, hypoglycemic, and antioxidant biological activities will be significantly enhanced (Kisku et al., 2022; Li et al., 2022; Malacaria et al., 2022; Raza et al., 2016; Yang et al., 2014). The potent chelating ability of flavonoids with several metal ions, including Zn (II), Cu (II), Fe (II), Cr (III), Co (II), Mn (II), and Pb (II), has been studied extensively. Jayaprakash et al. (2023); Kojik et al. (2021) Zinc is a trace element that is necessary for human health. It is crucial for growth and development, immunological response, brain function, reproduction, etc., (Cummings and Kovacic, 2009). The findings showed that the newly created compound Rutin-zinc (II) was a more effective antioxidant in vitro and displayed more significant anti-tumor ability on leukemia, multiple myeloma, and melanoma cell lines than free rutin (Ikeda et al., 2015). Another study revealed that the radical scavenging effect of the Iso-orientin-Zinc complex was substantially greater than that of iso-orientin (Wang et al., 2021). However, the preparation and antioxidant activity of the phlorizin-Zn (II) complex remains unclear.
In this study, single-factor testing and RSM were used to improve the phlorizin-Zn (II) complex preparation procedure, and the optimal preparation process with the best preparation rate was obtained. The antioxidant activity of the phlorizin-Zn (II) complex was determined using DPPH, ABTS, hydroxyl free radical scavenging, and FRAP test. This study will provide a deeper understanding of the functions of the phlorizin-Zn (II) complex and lay the groundwork for its future development and utilization.

Materials and Methods

Materials

Phlorizin (≥98%), DPPH, and ABTS were obtained from Yuanye Biotechnology Co., Ltd (Shanghai, China). Salicylic acid was purchased from Sigma-Aldrich (St Louis, MO, USA). More chemicals were ordered from Sinopharm Chemical Reagent Co., Ltd (Beijing, China) and were of analytical grade. Ultrapure water was prepared for the experiment.

Preparation of Phlorizin-Zn (II) Complex

The zinc acetate ((CH₃ COO)₂Zn) solution (0.1 moL/L) was gradually incorporated into phlorizin solution (0.1 moL/L) to achieve a molar ratio of 1:1 and the pH was adjusted to 6 by sodium acetate solution. The precipitate was collected by centrifugation following a 4 h reaction at 50°C and the ultrapure water and ethanol were then cleaned, respectively. Phlorizin-Zn (II) complex was obtained by freeze-drying. The preparation flow chart of the phlorizin-Zn (II) complex is shown in Fig. 1.

Single Factor Experimental Design

To examine the impact of several variables on the yield of phlorizin-Zn (II) complex, single-factor experiments were used. pHs were set at 5-9. Reaction temperatures were set at 30-70°C. Reaction times were set at 1-5 h and the molar ratios of phlorizin to metal ions were set at 1:1, 1:2, 1:3, 2:1, and 3:1.

Box-Behnken Design (BBD) for Phlorizin-Zn (II) Complex

A three-factor, three-level response surface analysis experiment was designed based on three factors that significantly affected the yield of the phlorizin-Zn (II) complex. The yield of the phlorizin-Zn (II) complex was taken as the response value (Sun et al., 2023). The amounts of coded and uncoded independent variables are shown in Table 1. A total of 17 experimental runs in random order were needed. The quadratic regression model was stated in its general form as follows (Kadam et al., 2023):

\[ Y = \beta_0 + \sum_{i=1}^{[3]} \beta_i X_i + \sum_{i=1}^{[3]} \sum_{i<j}^{[3]} \beta_{ij} X_i X_j + \sum_{i=1}^{[3]} \beta_{i2} X_i^2 \]  

(1)

where, \( Y \) is the predicted phlorizin-Zn (II) complexed yield; \( \beta_0 \) is the constant term. \( \beta_i \) is the regression coefficient of the linear term, \( \beta_{ij} \) is the regression coefficient of the quadratic term and \( \beta_{ij} \) is the regression coefficient of the interaction term. \( X_i \) and \( X_j \) are the coded independent variables.

DPPH Free Radical Scavenging Assay

DPPH solution of 100 μmol/L was prepared with anhydrous ethanol as solvent and stored in the dark. The phlorizin or phlorizin-Zn (II) complex solution (final concentration 0.5-5 mg/mL) of the same volume was mixed with DPPH solution and the reaction was completed at 298 K in a dark state for 30 min. Every group’s absorbance was calculated when the wavelength was 517 nm (Xiao et al., 2020). The DPPH radical scavenging ability of phlorizin and phlorizin-Zn (II) complex was determined using the following equation:

\[ \text{DPPH radical scavenging rate}(\%) = \left(1 - \frac{A_1 - A_3}{A_2}ight) \times 100\% \]  

(2)

where, \( A_1 \), \( A_2 \) and \( A_3 \) were the absorbance of the sample and DPPH solution, the sample without the DPPH solution, and the ethanol and DPPH solution, respectively.

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**Table 1:** The range of independent parameters in the design of the experiment on the phlorizin-Zn(II) complex

<table>
<thead>
<tr>
<th>Levels</th>
<th>-1</th>
<th>0</th>
<th>+1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (X₁)/h</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>pH (X₂)</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Temperature (X₃)/°C</td>
<td>40</td>
<td>50</td>
<td>60</td>
</tr>
</tbody>
</table>

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**Fig. 1:** The preparation flow chart of phlorizin-Zn (II) complex
**ABTS Free Radical Scavenging Assay**

ABTS solution (10 mL; 7 mmol/L) was thoroughly mixed with K$_2$S$_2$O$_8$ solution (5 mL; 7.35 mmol/L) and then the mixed solution was stored under light-free conditions for 16 h to obtain the ABTS stock solution. Then ABTS reserve solution was prepared by dilution with anhydrous ethanol and detected at 734 nm until the absorbance value of the ABTS working solution was 0.7±0.02. ABTS working solution (1 mL) was combined with different concentrations of phlorizin or phlorizin-Zn (II) complex solution (200 μL; final concentration 0.05-0.5 mg/mL) and the mixed solution was placed in the dark at room temperature for 6 min. Every group’s absorbance was calculated when the wavelength was 734 nm (Rumpf et al., 2023). The following formula was used to determine the ABTS radicals’ scavenging activity:

\[
ABTS \text{ radical scavenging ability}(%) = \left(1 - \frac{A_1}{A_2}\right) \times 100 \quad (3)
\]

where, $A_1$ is the absorbance of the ABTS working solution and different concentrations of samples. $A_2$ is the absorbance of distilled water mixed with ABTS working solution.

**Hydroxyl Radical Scavenging Assay**

The different complexation of phlorizin or phlorizin-Zn (II) complex solution (100 μL) was mixed with Fe$_2$SO$_4$ solution (200 μL, 1.8 mmol/L) and salicylic acid-ethanol solution (1 mL, 1.8 mmol/L) then H$_2$O$_2$ solution (10 μL, 0.03%) was added to the mixture. The mixture was kept at 310 K for 30 min. The absorbance value was measured at 510 nm wavelength (Sihag et al., 2022). The following formula was used:

\[
OH \text{ radical scavenging rate}(%) = \left(1 - \frac{A_i - A_j}{A_j}\right) \times 100\% \quad (4)
\]

where, $A_i$ is the absorbance of the mixture with the sample, Fe$_2$SO$_4$ solution, salicylic acid-ethanol solution, and H$_2$O$_2$ solution. $A_j$ is the absorbance of the mixture with sample and ultrapure water. $A_j$ is the absorbance of the mixture of ultrapure water, Fe$_2$SO$_4$ solution, salicylic acid-ethanol solution, and H$_2$O$_2$ solution.

**FRAP Assay**

Acetate buffer (100 mL; pH 3.5), TPTZ solution (2,4,6-tri (2-pyridyl)-s-triazine; 10 mL; 10 mmol/L) and ferric chloride solution (10 mL; 20 mmol/L) were mixed and placed at 310 K for 1 h to prepare the FRAP solution. The preheated FRAP solution (150 μL) was mixed evenly with ultrapure water (15 μL) and the phlorizin and phlorizin-Zn (II) complex solution (5 μL; final concentration 0.05-0.5 mg/mL). The mixed solution was then left at a temperature of 310 K for 30 min in the absence of light to allow for full reaction. The absorbance of the mixture was measured at 593 nm. Every group’s absorbance was calculated when the sample solution was replaced with FeSO$_4$ standard solution of different concentrations and the operation was repeated according to the above steps to draw the standard curve (Wootton-Beard et al., 2011).

**Statistical Analysis**

Analysis of Variance (ANOVA) was used to examine the data, which were reported as mean ± Standard Deviation (SD). Design expert software (10.0.7) was used for experimental design and statistical analysis.

**Results and Discussion**

**Single Factor Experiment for the Preparation of Phlorizin-Zn (II) Complex**

**Effect of Reaction pH on the Yield of Phlorizin-Zn (II) Complex**

The acid-base property of reaction solution has great influence on the degree of metal dissociation (An et al., 2022). Therefore, the pH value has a great influence on the coordination reaction during the test. The yields of phlorizin-Zn (II) complex at different pH were measured and shown in Fig. 2(a). When the pH increased, the yield of phlorizin-Zn (II) complex increased continuously, however when the pH was greater than 6, the yield of phlorizin-Zn (II) complex began to decrease. Meanwhile the yield of the phlorizin-Zn (II) complex under acidic conditions was higher than under alkaline conditions. The possible cause was that metal ions were more easily dissociated under acidic conditions, which was conducive to the progress of the reaction. The highest yield of the phlorizin-Zn (II) complex reached 85.25% when the pH of the reaction solution was 6. Other study revealed that the changes of pH affected the formation of tea polysaccharide (TPS) complexes and TPS complexes were more easily formed when the reaction pH was close to the pH of the TPS solution (Fan et al., 2021).

**Effect of Reaction Time on the Yield of Phlorizin-Zn (II) Complex**

The reaction time of flavonoids and metal ions is directly related to whether the coordination reaction is complete. Fig. 2(b) showed that the yields of phlorizin-Zn(II) complex at different reaction time. The yield of phlorizin-Zn (II) complex progressively increased and then decreased as the extension of reaction time and the
yield got the max (79.01%) at 4 h. The reason might be that when the reaction time was short, the reaction of zinc acetate and phlorizin was insufficient. Then when a generation of the complex reached saturation in the reaction after a long time, the reverse reaction was greater than the forward reaction, which resulted in a decrease in the yield of the phlorizin-Zn (II) complex. In other research, the reaction time in the preparation of gallic acid metal complex was 3 h (El-Megharbel and Hamza, 2022). It could be seen that different flavonoids-metal complex had different preparation times.

**Effect of Reaction Temperature on the Yield of Phlorizin-Zn (II) Complex**

Temperature has an important impact on the rate of the chemical reaction (Eliason and McMahon, 1981). The rise of temperature often encourages the coordination reaction between flavonoids and metal ions. The yields of phlorizin-Zn (II) complex at different reaction temperatures were measured as Fig. 2(c) illustrates. As the temperature raised the yield of the phlorizin-Zn (II) complex increased. The yield of the phlorizin-Zn (II) complex reached the maximum of 79.98% at 50°C. However, with a further increase in temperature, the yield of the phlorizin-Zn (II) complex began to decrease. The 55°C was selected as the reaction temperature when prepared the iso-orientin-metal complex, which was similar to our research (Wang et al., 2021). The explanation might be that as the temperature rose, the chelating ability of phlorizin and zinc ions continued to increase, resulting in the continuous increase of yield of the phlorizin-Zn (II) complex. However, with the further increase in temperature, the thermal stability of phlorizin was affected and the molecular structure of phlorizin was destroyed by long heat treatment.

**Effect of the Molar Ratio on the Yield of Phlorizin-Zn (II) Complex**

Figure 2(d) illustrates that the influence of the molar ratio on the yield of the phlorizin-Zn (II) complex. The yields of phlorizin-Zn (II) complex were no significant difference in the dosage ratio of different molar ratios. So, we chose the molar ratio (phlorizin: Zn²⁺) of 1:1 for follow-up experiments.
Table 2: Optimization of preparation parameters for phlorizin-Zn(II) complex by response surface design

<table>
<thead>
<tr>
<th>Run</th>
<th>X₁ (h)</th>
<th>X₂</th>
<th>X₃ (°C)</th>
<th>Y (%)</th>
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<td>1</td>
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<td>60</td>
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<td>2</td>
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<td>17</td>
<td>5</td>
<td>5</td>
<td>50</td>
<td>71.21</td>
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Optimization of the Preparation Process by RSM

Experimental Design and Result Analysis by RSM

Based on the single-factor test, pH, time, and temperature were taken as test factors to conduct RSM. Table 2 lists the three components, their levels, and the RSM results. Multiple regression analysis using the experimental data obtained, the quadratic regression equation of the actual values of the factors in this model was obtained. The relationship between preparation yield and the independent variable was verified by a mathematical model. The final equation based on the coding factor was as follows:

\[
Y = 185.90 + 1.19375X_1 - 0.1337X_2 - 1.317X_3 + 1038X_1X_2 + 1.525X_1X_3 + 1.1625X_2X_3 - 5.699X_1^2 - 1.5445X_2^2 - 5.147X_3^2
\]

\[r^2_{\text{adj}} = 0.9756, \quad r^2_{\text{pred}} = 0.9012\]

where, \(Y\) is the yield of the phlorizin-Zn(II) complex. \(X_i\) is the coded variable for time, \(X_2\) is the coded variable for PH and \(X_3\) is the coded variable for temperature.

Table 3 presented the results of the experimental analysis of variance, the model (p<0.0001) was sky-high significant. According to statistics, the model's misfit was not significant (p>0.05). The trial's predicted and actual values were determined to be well-matched by the \(R^2\) value of 0.9815. The adjusted coefficient of determination \(r^2_{\text{adj}} = 0.9756\) and the prediction coefficient of determination \(r^2_{\text{pred}} = 0.9012\) were similar \((r^2_{\text{adj}} - r^2_{\text{pred}} < 0.2)\), indicating that this regression equation was reliable and could accurately reflect the relationship of phlorizin-Zn(II) complex yield between response values and various experimental factors (Akalin et al., 2015; Cheng et al., 2020; Hao et al., 2023).

The F-value and p-value of each model term were used as tools to evaluate each parameter's importance. The magnitude of the F-value was proportional to the effect of this factor on the response surface (Hatami et al., 2023; Latif et al., 2022). Table 3 demonstrated that the yield of the phlorizin-Zn(II) complex was significantly influenced (p<0.05) by linear coefficients \((X_i, X_2)\), cross coefficients \((X_1X_2, X_1X_3, X_2X_3)\), and quadratic coefficients \((X_i^2, X_2^2, X_3^2)\). While there was no significant coefficient of other terms (p>0.05). Therefore, the reaction temperature \((X_3)\) and reaction time \((X_2)\) followed by pH \((X_1)\) were the main factors affecting the phlorizin-Zn(II) complex yield.

Table 3: Regression coefficients and ANOVA estimated for phlorizin-Zn(II) complex yield

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of squares</th>
<th>Df</th>
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<th>F-value</th>
<th>p-value</th>
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<td>X₁</td>
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<td>11.040</td>
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<tr>
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<td>0.014</td>
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<tr>
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<td>13.089</td>
<td>16.08</td>
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<tr>
<td>X₁X₂</td>
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<td>01</td>
<td>7.062</td>
<td>8.82</td>
<td>0.0208 *</td>
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<tr>
<td>X₁X₃</td>
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<td>01</td>
<td>0.093</td>
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<td>X₂X₃</td>
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<tr>
<td>X₁²</td>
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<td>Cor Total</td>
<td>326.016</td>
<td>16</td>
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</table>

\(R^2 = 0.9815\)

***, the difference is significant at 0.05 level (p<0.05); **, the difference is significant at 0.01 level (p<0.01); ns, the difference is not significant (p>0.05)
Fig. 3: Response surface plots and contour plots for interaction between various factors on the yield of phlorizin-Zn (II) complex, time and pH (a); Time and temperature (b); and pH and temperature (c)
optimizing independent parameters such as pH, temperature, and time. Figure 4 demonstrated that there was no discernible difference between the predicted and actual values. The optimal preparation scheme of the phlorizin-Zn (II) complex was obtained by design expert software analysis as follows: The time of 4.097 h, the pH of 5.951, the temperature of 48.678°C and the molar ratio (phlorizin: Zn$^{2+}$) of 1:1. Under these conditions, the predicted preparation yield of phlorizin-Zn (II) complex was 79.052%. Considering the feasibility of actual operation, the optimal conditions given by the model were rounded, so the optimal preparation conditions included: The time of 4.1 h, the pH of 5.9, the temperature of 48.7°C, and the molar ratio (phlorizin: Zn$^{2+}$) of 1:1. Under these experimental circumstances, the verification test was conducted and the average preparation yield was determined to be 78.02% through three parallel determinations, which quite closed to the predicted value. The outcomes demonstrated the model's dependability and suitability for use as a guide in determining the precise process parameters.

The Antioxidative Activity of Phlorizin-Zn (II) Complex

DPPH Free Radical Scavenging Activity

Determining the DPPH free radical scavenging capacity is a typical way to assess the antioxidant potential of natural products and bioactive substances since DPPH is a free radical that is quite stable (Alamgir et al., 2014). Figure 5(a), the DPPH free radical scavenging rates of phlorizin and phlorizin-Zn (II) complex were dose-dependent within the concentration range of 0.5-5 mg/mL. When the dosage was 5 mg/mL, the DPPH free radical scavenging rates of phlorizin and phlorizin-Zn (II) complex were 57.16 and 74.21% respectively. Thus, The DPPH scavenging ability of the phlorizin-Zn (II) complex was higher than that of phlorizin. Quercetin-iron complexes were substantially more effective in scavenging DPPH radicals than free quercetin. This demonstrates that quercetin-iron complexes were far stronger antioxidants than quercetin (Raza et al., 2016). Flavone's potent antioxidant qualities are attributed to its many binding sites. In addition, metal ions also have antioxidant activity (Van Acker et al., 1996). Therefore, by chelating metal ions, flavonoid metal complexes could better prevent the production of free radicals and their subsequent reactions to improve antioxidant activity (Wang et al., 2021).

ABTS Free Radical Scavenging Activity

The greatest absorption peak of the blue-green ABTS$^+$ free radical solution was 734 nm. When the interaction occurs, the color of the ABTS$^+$ radical solution gradually becomes lighter and when the phenomenon of color change exists, it represents the ability to have antioxidant activity (Marc et al., 2004). In a dose-dependent manner, phlorizin and phlorizin-Zn (II) complex scavenged ABTS free radicals (Fig. 5(b)).
When the dosage was 0.05-0.5 mg/mL, the ABTS scavenging rate of phlorizin-Zn (II) complex was stronger than that of phlorizin. When the dosage was 0.4 mg/mL, the ABTS free radical scavenging capacity of the phlorizin-Zn (II) complex was 11% higher than that of phlorizin (p<0.05). Flavonoid metal complexes maintain all flavonoid properties and their pharmacokinetic characteristics are enhanced after being combined with metal ions (Kasprzak et al., 2015). It was discovered that following the coordination process between metal ions and flavonoids, hyper-delocalized structures with more active sites were formed and these active sites further improved the metal-chelated flavonoids' capacity to scavenge free radicals (Zhang et al., 2020).

Fig. 5: Inhibition effects of phlorizin-Zn (II) complex on DPPH radicals (a); ABTS radicals (b); Hydroxyl radicals (c); and reducing power (d); *p<0.05, **p<0.01 vs phlorizin group

Hydroxyl Radical Scavenging Activity

The most hazardous and poisonous active oxygen-free radical that organisms are aware of is hydroxyl (Yu et al., 2021). High reactive hydroxyl free radical has strong oxidation on potassium chromate and potassium permanganate and was the three-electron reduction product of oxygen. It interacts with a variety of molecules in the biological body through hydrogen extraction, addition, or electron transfer, causing oxidation and damage to proteins, amino acids, nucleic acids, and lipids, finally resulting in cell necrosis or mutation (Purushothaman et al., 2020). Hydroxyl radicals were also associated with tumor, phagocytosis, senescence, and radiation damage (Rafat Husain et al., 1987). Figure 5(c) showed that both phlorizin and phlorizin-Zn (II) complex had a strong scavenging effect on hydroxyl radicals and the scavenging abilities were proportional to their concentrations. However, the scavenging activity of phlorizin was lower than that of the phlorizin-Zn (II) complex at the same concentration. The highest scavenging rate of the phlorizin-Zn (II) complex reached 70.22% and its IC50 was 0.293 mg/mL. The stronger hydroxyl free radical scavenging ability of phlorizin-Zn (II) complex than free phlorizin might be ascribed to the synergistic effect of phlorizin and Zn2+ which promoted the contact between phlorizin-Zn (II) complex and hydroxyl free radical and then improved the antioxidant property of phlorizin-Zn (II) complex (Zhang et al., 2020). Previous reports also found that the Iso-orientin-Zinc complex had a greater hydroxyl radical scavenging capacity than iso-orientin in a certain concentration range (Wang et al., 2021).

FRAP Assay

In the FRAP Assay, the total antioxidant substance in the sample reduces Fe3+ to Fe2+, then Fe2+ and tripyridyl-triazine form a blue-purple complex with strong absorption at 593 nm wavelength. Thus FRAP assay not
only reflects the scavenging activity of a certain free radical but also reflects the total antioxidant capacity of the sample (Bolanos De et al., 2015; Jones et al., 2017). The higher FeSO₄ concentration in the reaction mixture indicates the more significant reduction capacity of antioxidants. Figure 5(d), at the 0.05-0.5 mg/mL concentration range, the FeSO₄ concentration increased gradually with the increase of phlorizin and phlorizin-Zn (II) complex concentration, indicating that the FRAP activities of phlorizin and phlorizin-Zn (II) complex were gradually enhanced. At the same concentration, the FRAP activity of the phlorizin-Zn (II) complex was better than phlorizin. When the concentration was 0.5 mg/mL, the FRAP activity of the phlorizin-Zn (II) complex was 23.9% which was higher than that of phlorizin. The results demonstrated the electron-donating property of the phlorizin-Zn (II) complex for neutralizing free radicals and the formation of stable products. Another study also showed that the ability of the metal curcumin complex to reduce iron ions is stronger than free curcumin (Joshi et al., 2023). In summary, the FRAP of the phlorizin-Zn (II) complex was stronger than that of free phlorizin.

**Conclusion**

In this study, the preparation technology of the phlorizin-Zn (II) complex was optimized. The results showed that the maximum yield of phlorizin-Zn (II) complex was 78.02%, which was quite close to the predicted value, while the parameters at this time were 4.1 h, pH 5.9, temperature 48.7°C and the molar ratio (phlorizin: Zn²⁺) was 1:1, respectively. Moreover, the phlorizin-Zn (II) complex had stronger free radical scavenging ability and reducing power than phlorizin. The synergistic effect of phlorizin and Zn²⁺ improved the antioxidant property of the phlorizin-Zn (II) complex. More importantly, future work will focus on the mechanism of its functional action.

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**Author’s Contributions**

**Yuqing Zhang:** Participate in the whole experimental designed, experimental method, result analysis and manuscript written.

**Yaping Li:** Participate in part of the experimental process and the analysis of results.

**Guorui Yang:** Participated in part of the experiment.

**Shaoxuan Yu:** Ameliorated the manuscript.

**Haifang Xiao:** Participated in the experiment designed guidance, result interpretation, and ameliorated the manuscript.

**Yuanda Song:** Participated in part of the guidance of experimental designed and ameliorated the manuscript.

**Ethics**

The original content in this piece hasn't been released before. The corresponding author attests that there are no ethical concerns and that every other author has read and approved the paper.

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