Optimization of ultrasound-assisted enzymatic extraction and in vitro antioxidant activities of polysaccharides extracted from the leaves of *Perilla frutescens*

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Abstract

A Box-Behnken design was employed to optimize ultrasound-assisted enzymatic extraction of polysaccharides from perilla leaves. Four independent variables, namely, liquid-to-solid ratio, enzymatic time, enzymatic temperature, and ultrasonic power were investigated. Under the optimal liquid-to-solid ratio of 41:1, enzymatic time of 40 min, enzymatic temperature of 49 °C, and ultrasonic power of 204 W, the experimental polysaccharide yield was 3.84% (n = 3), which was close to the value predicted by response surface methodology (3.9%). Four polysaccharides (PLP1, PLP2, PLP3, and PLP4) were obtained with DEAE-cellulose-52 and Sephadex G-100 chromatography, and their antioxidant activities were investigated with various antioxidant assays *in vitro*. All the four polysaccharides possessed good antioxidant properties in a concentration-dependent manner, and PLP3 presented the highest antioxidant activity. Thus, the novel polysaccharides extracted from perilla leaves could be promising bioactive macromolecules that can be applied as potential antioxidants in medical and food industries.

Keywords: perilla leaves; polysaccharide; ultrasound-assisted enzymatic extraction; antioxidant activities.

Practical Application: The study provides valuable information that ultrasound-assisted extraction is an efficient and green process for the preparation of extracts from perilla leaves, and will help in the development of a naturally potential antioxidant for pharmaceutical and food industries.

1 Introduction

*Perilla frutescens* (L.) Britton, an annual herbaceous plant belonging to the Labiatae family (Peiretti et al., 2011), is an important cash crop widely used for cooking and medicinal purposes in several Asian countries such as China, Korea, Japan, and Thailand (Li et al., 2015). The leaves of *P. frutescens*, usually accompanied with sea foods, are believed to prevent food poisoning and protect the digestive tract from inflammatory diseases. In addition, perilla leaves have been widely used as a diuretic, a sedative, an antioxidant, and an antifebrile in traditional medicine (Kwon et al., 2002). To date, more than 100 compounds, including essential oils, phenolic acids, flavones, terpenoids, polysaccharides, sterols, and other constituents, have been isolated and identified from perilla leaves. Pharmacological studies had demonstrated the enormous medicinal potentials of perilla leaves, including detoxicant, antitussive, antipyrethic, and antibiotic properties (Liu et al., 2013).

Polysaccharides have been extensively studied as additives in food and pharmaceutical industries owing to their unique bioactive properties and chemical structures (Liao et al., 2015). In particular, polysaccharides from plants possess several biological activities, including antitumor (Zhu et al., 2011), antioxidation (Souza et al., 2012; Tian et al., 2012), anti-inflammatory (Chen et al., 2012; Wang et al., 2015), antibacterial, antifungal, and antiviral activities (Komatsu et al., 1997). Although several methods are available for the extraction of plant polysaccharides, including organic solvent extraction, enzyme-assisted extraction, microwave-assisted extraction, ultrasonic extraction, and supercritical fluid extraction (Wang et al., 2015; Hu et al., 2016), to the best of the authors’ knowledge, there are only very few reports on the optimization of extraction of polysaccharides from perilla leaves. Enzyme-assisted extraction is the principal and most conventional extraction method, requiring a long extraction period and high temperature (Le & Le, 2012). In contrast, ultrasonic extraction is one of the recent extraction techniques, which has been largely studied for processing of food products like dairy products aiming to avoid the negative effects of the conventional thermal processing, like protein denaturation, vitamin and lactose degradation, among others (Monteiro et al., 2018; Guimarães et al., 2018). The ultrasound technology has several advantages, including high reproducibility in shorter time, simplified manipulation, reduced solvent consumption and temperature, lower energy input, and easy scale-up for industrial applications (Tabaraki & Nategh, 2011; Tao et al., 2014; Li et al., 2016; Um et al., 2017). Thus, conventional enzyme-assisted extraction method coupled with ultrasound irradiation may be an effective method for polysaccharides extraction from perilla leaves.

Response surface methodology (RSM), which has been widely used in recent times for the optimization of extraction methods, is generally considered as an effective statistical technique for optimizing complex processes (Liu et al., 2015). This technique can reflect the complete effects of variables and efficiently simplify the experimental procedures (Chen et al., 2015a; Wu et al., 2013). In the present study, polysaccharides from perilla leaves were extracted using ultrasound-assisted...
enzymatic extraction (UAE) method, and RSM was applied with a Box-Behnken design (BBD) to optimize the extraction parameters (e.g., liquid-to-solid ratio, enzymatic temperature, enzymatic time, and ultrasound power). In addition, the in vitro antioxidant activity of the purified polysaccharides (based on reducing power) were evaluated with reducing power, 2,2'-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl (•OH), and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays.

2 Materials and methods

2.1 Plant materials

P. frutescens leaves were collected in September 2017 from North University of China (Taiyuan, Shanxi Province, China), air-dried, ground into powder using a grinder, and passed through a 40-mesh sieve. All the chemicals used in the experiment were of analytical grade.

2.2 UAE

The crude polysaccharides extraction from perilla leaves by ultrasonic-assisted process was performed in a tunable ultrasonic bath (TH-400BQG, 50 kHz, 200 W; Tianhua Ultrasound Electronic Equipment Co., Jining, Shandong, China). A total of 5 g of dried perilla leaves powder were suspended in 50 mL of water and extracted with different amounts of cellulase. After UAE, the extracted solutions were centrifuged at 12,000 × g for 10 min, filtered, and the supernatants were concentrated using a rotary evaporator (SHZ-95B, Yuhua Ltd., China). The concentrates were precipitated with dehydrated ethanol, incubated for 12 h at 4°C, and centrifuged at 12,000 x g for 10 min. Then, the precipitates were washed with dehydrated ethanol, dissolved in water, and dialyzed against 100 volumes of water. The dialysate was freeze-dried to obtain crude polysaccharides. The polysaccharides were measured by the phenol-sulfuric acid method with glucose as the standard (Dubois et al., 1956), and the polysaccharides yield (%) was calculated as follows:

\[
\text{Polysaccharides yield (\%) = \frac{\text{weight of crude polysaccharides (g)}}{\text{weight of sample (g)}} \times 100\%}
\]  

(1)

2.3 Single factor experiments

The effects of liquid-to-solid ratio (20, 30, 40, 50, and 60 (mL:g), respectively), cellulase content (cellulase weight per material weight, 0.5%, 1.0%, 1.5%, 2.0%, and 2.5%, respectively), enzymatic time (20, 30, 40, 50, and 60 min, respectively), enzymatic temperature (30°C, 40°C, 50°C, 60°C, and 70°C, respectively), and ultrasonic power (120, 160, 200, 240, and 280 W, respectively) on polysaccharides yield were studied based on single-factor design, with one factor being changed and the other factors being kept constant in each experiment.

2.4 RSM design and statistical analysis

A three-level-four-factor BBD model was adopted to determine the optimal conditions for the UAE. The effect of four independent variables, i.e., liquid-to-solid ratio (X1), enzymatic time (X2), enzymatic temperature (X3), and ultrasonic power (X4), on the polysaccharides yield (Y) were tested in a 29-run experiment. Five replications of the central points were used to evaluate the pure error. The experimental data (Table 1) were analyzed using multiple regressions to fit the following quadratic polynomial model:

\[
Y = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \beta_{ii} X_i^2 + \sum_{i=1}^{4} \sum_{j=1}^{4} \beta_{ij} X_i X_j
\]

(2)

where Y is the response variable (polysaccharides yield); \(\beta_0\), \(\beta_i\), \(\beta_{ii}\), and \(\beta_{ij}\) are the offset term, linear effect, quadratic effect, and interaction effect, respectively. The coded independent variables. Analysis of the experimental data and optimized conditions was performed using Design Expert software (Version 8; Stat-Ease, Inc., Minneapolis, MN, USA).

2.5 Purification of the crude polysaccharides

The crude polysaccharides from perilla leaves were resolved for the removal of proteins by using the Sevag method (Sevag et al., 1938), and purified by employing DEAE-52 cellulose chromatography column (2.6 × 30 cm) equilibrated with distilled water. The column was eluted with different concentrations of sodium chloride solution (0, 0.1, 0.2, 0.3, and 0.5 mol/L) at a flow rate of 0.6 mL/min. The eluates (10 mL/tube) were collected and ascertained using the phenol-sulfuric acid method. Finally, the main fractions (PLP1, PLP2, PLP3, and PLP4) were obtained, dialyzed, and lyophilized for further investigation of antioxidant activities.

2.6 Antioxidant activities

The reducing power of the polysaccharide fractions was determined according to the method of Oyaizu (1986). The reaction mixture containing 2.5 mL of the sample at different concentrations (0.1, 0.2, 0.4, 0.8, 1.0, 2.0, 3.0, 4.0, and 5.0 mg/mL), 2.5 mL of 0.1 M sodium phosphate buffer (pH 6.6), and 2.5 mL of K3Fe(CN)6 (1%, w/v) was incubated at 50°C for 20 min. Then, 2.5 mL of trichloroacetic acid (10%, w/v) were added, and the mixture was centrifuged at 7200 g for 10 min. Subsequently, 2.5 mL of the supernatant were mixed with 2.5 mL of deionized water and 0.5 mL of FeCl3 (0.1%, w/v) in a test tube, and the absorbance was measured at 700 nm. Ascorbic acid was used as the positive control. Increased absorbance values indicated a higher reducing power. DPPH scavenging activity was assessed using the method of Choi et al. (2000). The •OH scavenging activity was examined as previously described by De Avellar et al. (2004). The ABTS scavenging activity was measured as reported previously (Cui et al., 2017).
UAEE of polysaccharides from perilla leaves

was chosen as the optimal liquid-to-solid ratio for the extraction of polysaccharides from perilla leaves.

Figure 1B shows the effect of cellulase content on the yield of polysaccharides. It was noted that the yield of polysaccharides was significantly increased with increasing cellulase content up to 2.0%, and thereafter slightly decreased with further increase in cellulase content. To avoid wastage of cellulase, 2.0% was chosen as the optimum cellulase content.

At the enzymatic time of 20–40 min, the yield of polysaccharides increased, reaching a maximum value at 40 min (Figure 1C). However, with prolonged extraction time, the polysaccharides yield slightly decreased, suggesting that the polysaccharides had been completely extracted, a phenomenon similar to that reported in a previous study (Zheng et al., 2014). As prolonged duration can cause energy wastage and increase in production cost (Wu et al., 2013), 40 min was selected as the optimal enzymatic time.

The yield of polysaccharides sharply increased when the enzymatic temperature was increased from 30 °C to 50 °C, and reached the maximum value (3.46%) at 50 °C, as shown in Figure 1D. However, the polysaccharides yield started to decrease with further increase in enzymatic temperature. The low yield at high temperature could partly be explained by the loss of enzyme activity due to thermal inactivation (Chen et al., 2015b). Thus, 50 °C was selected as the optimal enzymatic temperature for the extraction of polysaccharides from perilla leaves.

The effect of ultrasonic power on the yield of polysaccharides is shown in Figure 1E. With increasing ultrasonic power (120–200 W), the polysaccharides yield increased, reaching a maximum value at 200 W. At ultrasonic power higher than 200 W, the yield of polysaccharides slowly declined, which might be owing to the degradation of polysaccharides as a result of excessive ultrasonic power (Li et al., 2012). Therefore, 200 W was chosen as the optimum ultrasonic power for the extraction of polysaccharides from perilla leaves.

3.2 Fitting the mathematical model

Design-Expert (Version 8.06) software was adopted to analyze the experimental data, and the ANOVA results are shown in Table 2. It must be noted that the significance of regression coefficient increased with the increasing $F$ value and decreasing $P$ value (Liao et al., 2015). In the present study,
an F value of 84.58 and low P value (<0.0001) suggested that the response surface quadratic model was significant, and the probability of error in the model F value was 0.01%. Meanwhile, the determination coefficient (R²) was 0.9883, indicating that 98.83% of the variation could be explained by the fitted model. The adjusted determination coefficient (adj. R² = 0.9766) also revealed that < 2.4% of the total variations could not be explained by the model. In addition, a P value of 0.063 implied that the lack of fit was not significant (P> 0.05) relative to the pure error. Adeq Precision measures the signal-to-noise ratio, and a ratio of > 4 is desirable. Thus, the ratio of 27.74 indicated adequate signal and the capability of the model for navigating the design space. The results revealed that the response variable and independent variables were related by the following quadratic polynomial equation:

\[
Y = 3.89 + 0.14X_1 + 0.038X_2 - 0.092X_3 + 0.17X_4 + 0.23X_1X_2 + 0.20X_1X_3 + 0.18X_1X_4 - 0.34X_2X_3 - 0.063X_2X_4 + 0.083X_3X_4 - 0.62X_1^2 - 0.66X_2^2 - 0.60X_3^2 - 0.89X_4^2
\]

where Y is the yield of polysaccharides (%), \(X_1\) is the liquid-to-solid ratio (mL:g), \(X_2\) is the enzymatic time (min), \(X_3\) is the enzymatic temperature (°C), and \(X_4\) is the ultrasonic power (W).

Figure 1. Effect of (A) liquid-to-solid ratio, (B) cellulase content, (C) enzymatic time, (D) enzymatic temperature, and (E) ultrasonic power on the polysaccharides yield of perilla leaves.
Table 2. Analysis of variance (ANOVA) for the quadratic polynomial mode.

| source         | Sum of squares | df | Mean square | F value | P-value* |
|----------------|----------------|----|-------------|---------|----------|
| Model          | 10.05          | 14 | 0.718       | 84.58   | < 0.0001 |
| $X_1$          | 0.221          | 1  | 0.221       | 26.09   | 0.0002   |
| $X_2$          | 0.018          | 1  | 0.018       | 2.078   | 0.1714   |
| $X_3$          | 0.103          | 1  | 0.103       | 12.10   | 0.0037   |
| $X_4$          | 0.354          | 1  | 0.354       | 41.68   | < 0.0001 |
| $X_1 X_2$      | 0.207          | 1  | 0.207       | 24.40   | 0.0002   |
| $X_1 X_3$      | 0.152          | 1  | 0.152       | 17.93   | 0.0008   |
| $X_1 X_4$      | 0.130          | 1  | 0.130       | 15.27   | 0.0016   |
| $X_2 X_4$      | 0.462          | 1  | 0.462       | 54.50   | < 0.0001 |
| $X_1 X_2 X_3$  | 0.016          | 1  | 0.016       | 1.842   | 0.1963   |
| $X_1 X_2 X_4$  | 0.027          | 1  | 0.027       | 3.209   | 0.0949   |
| $X_2^2$        | 2.472          | 1  | 2.472       | 291.3   | < 0.0001 |
| $X_3^2$        | 2.813          | 1  | 2.813       | 331.6   | < 0.0001 |
| $X_4^2$        | 2.334          | 1  | 2.334       | 275.1   | < 0.0001 |
| $X_1^2$        | 5.150          | 1  | 5.150       | 607.0   | < 0.0001 |
| Residual       | 0.119          | 14 | 0.008       |         |          |
| Lack of fit    | 0.110          | 10 | 0.011       | 5.203   | 0.0630   |
| Pure error     | 0.008          | 4  | 0.002       |         |          |
| Cor total      | 10.17          | 28 |             |         |          |
| $R^2$          | 0.9883         |    |             |         |          |
| Adj.$R^2$      | 0.9766         |    |             |         |          |
| Adeq precision | 27.74          |    |             |         |          |

*P < 0.01 highly significant; 0.01 < P < 0.05 significant; P > 0.05 not significant.

Analysis of the $F$ and $P$ values (Table 2) revealed that the effects of $X_1$, $X_2$, $X_3$, $X_4$, $X_1 X_2$, $X_1 X_3$, $X_1 X_4$, $X_2 X_3$, $X_2^2$, $X_3^2$, and $X_4^2$ on the polysaccharides yield were highly significant ($P < 0.01$). In contrast, $X_1 X_2 X_3$, and $X_1 X_4$ had no significant effects ($P > 0.05$) on the polysaccharides yield.

### 3.3 Analysis of the response surface

Figure 2 shows the three-dimensional response surface plots and their respective contour plots. By using these plots, the presence of any interactions between two variables can be easily assessed and the optimum values of the variables can be determined (Liao et al., 2015). In the present study, the polysaccharides yield was obtained along with two continuous variables in the response surface plots, while the other two variables were constant at their respective zero levels.

Figure 2A illustrates the effects of liquid-to-solid ratio and enzymatic temperature on the polysaccharides yield. With the increasing liquid-to-solid ratio from 30:1 to 41:1, the polysaccharides yield also increased. As the curve did not level off at low ratio, this liquid-to-solid ratio was well below the optimum for polysaccharides yield. There was an obvious increase in the polysaccharides yield as the enzymatic temperature was increased from 35 to 42 min; however, a further increase in the enzymatic temperature resulted only in slight change in polysaccharides yield. The effect of extraction time was less significant than that of liquid-to-solid ratio on the polysaccharides yield.

The effect of liquid-to-solid ratio and enzymatic temperature on the yield of polysaccharides is shown in Figure 2B. The interaction between these two variables and their quadratic variables had significant effects on the polysaccharides yield ($P < 0.01$). The polysaccharides yield evidently increased with the increase in enzymatic temperature from 45 °C to 50 °C; however, beyond 50 °C, the yield decreased. The low polysaccharides yield at high temperature could partly be explained by the loss of enzyme activity owing to thermal inactivation (Chen et al., 2015b).

According to Figure 2C, the interaction between liquid-to-solid ratio and ultrasonic power was significant ($P < 0.01$), and the polysaccharides yield increased with the increasing liquid-to-solid ratio and achieved optimal values at 39:1–41:1 and then decreased. Similarly, the yield of polysaccharides obviously increased when the ultrasonic power increased from 160 to 210 W, but beyond 210 W, the yield decreased with the increase of ultrasonic power.

From Figure 2D, it can be concluded that the interaction between enzymatic time and ultrasonic power had significant effects on the polysaccharides yield ($P < 0.01$). The yield of polysaccharides decreased with the ultrasonic power increased and reached maximum levels at 38–42 min and 45–48 °C, and a further increase in enzymatic time and enzymatic temperature led to a decrease in polysaccharides yield. The lower polysaccharides yield at higher temperatures and longer reaction periods might probably be owing to the decomposition of polysaccharides into monosaccharides and other small molecules (Liu et al., 2015; Wang & Lu, 2014). This result is consistent with those obtained for polysaccharides from the rhizome of Ligusticum chuanxiong (Liu et al., 2015).

The effects of enzymatic time and ultrasonic power on the polysaccharides yield are illustrated in Figure 2E. The linear and quadratic effects of ultrasonic power were significant.
(P < 0.01), indicating that an increase in ultrasonic power favored polysaccharides extraction at 160-210 W. At higher ultrasonic power, the polysaccharides yield decreased, which might be due to partial degradation of polysaccharides, indicating that a suitable ultrasonic power is important for polysaccharides extraction. Furthermore, maximum polysaccharides yield was achieved at 40-42 min, and a further increase in enzymatic time resulted in a decrease in polysaccharides yield.

According to Figure 2F, the polysaccharides yield increased with the increasing enzymatic temperature and ultrasonic power up to a certain threshold level, beyond which, the yield decreased, suggesting that suitable enzymatic temperature and ultrasonic power improve target compound solubility, solvent diffusion rate, and mass transfer (Hossain et al., 2011).

3.4 Verification of predictive model

The second-order polynomial equation was tested by using the selected optimal conditions, and the predicted optimum conditions for polysaccharides yield were as follows: liquid-to-solid ratio of 41.26:1, enzymatic time of 40.63 min,
enzymatic temperature of 49.33 °C, and ultrasonic power of 204.16 W. The predicted maximum polysaccharides yield was 3.9%. However, considering the requirements during actual production, the predicted optimum conditions were slightly modified as follows: liquid-to-solid ratio of 41:1, enzymatic time of 40 min, enzymatic temperature of 49 °C, and ultrasonic power of 204 W. Under these conditions, an average polysaccharide yield of 3.84% (n = 3) was obtained, which was well-matched with the predicted value. These results confirmed that the RSM model was adequate for the optimization of the extraction process (Xu et al., 2013).

3.5 Purification of perilla polysaccharides

The crude polysaccharides extract was purified by using DEAE-52 cellulose chromatography. The main polysaccharide fractions, named as PLP1, PLP2, PLP3, and PLP4, were eluted with 0, 0.1, 0.2, and 0.3 mol/L sodium chloride solution, respectively (Figure 3). Subsequently, the polysaccharide fractions were further purified using Sephadex G-100 column and eluted with distilled water. All the fractions formed a single peak, suggesting that they were homogeneous polysaccharides. The four fractions were collected, concentrated, dialyzed with water, and lyophilized for further examination of their antioxidant activities. The yields of PLP1, PLP2, PLP3, and PLP4 based on crude polysaccharides were 31.3%, 10.2%, 22.6%, and 12.8%, respectively.

3.6 Reducing power

The reducing power of a compound serves as an important indicator to evaluate its potential antioxidant activity, and the presence of antioxidants in the compound would result in the reduction of Fe$^{3+}$ to Fe$^{2+}$ by donating an electron (Zheng et al., 2014). The reducing powers of all the four polysaccharide fractions are shown in Figure 4A. It can be clearly observed from the figure that the reducing powers of PLP1, PLP2, and PLP3 increased with increasing sample concentration, while that of PLP4 increased gradually. The reducing powers of the four polysaccharide fractions were as follows: PLP3>PLP2> PLP1>PLP4. It must be noted that ascorbic acid, used as the positive control, presented a reducing power of 1.178 at 1.0 mg/mL, and the reducing powers of perilla polysaccharides were lower than that of ascorbic acid. These results revealed that the purified polysaccharide fractions extracted from perilla leaves had potent reducing power in vitro, and that PLP3 might contain more reductone-associated groups than PLP1, PLP2, and PLP4.

3.7 DPPH scavenging assay

DPPH is a stable free radical, which has been widely employed to evaluate the free radical scavenging activity of natural compounds (Zheng et al., 2014; Wu et al., 2013). Figure 4B illustrates the scavenging activities of the purified perilla polysaccharides against the DPPH radical, with ascorbic acid as the control standard. The DPPH scavenging activity increased with the increasing ascorbic acid concentrations from 0.1 to 1.0 mg/mL, reaching a maximum of 97.19% at 1.0 mg/mL. In contrast, the maximum DPPH scavenging activity of PLP3 was 72.61% at a concentration of 5.0 mg/mL. The IC$_{50}$ (defined as the concentration of a compound at which the scavenging rate reaches 50%) values for the DPPH scavenging activities of PLP1, PLP2, PLP3, and PLP4 were 5.3, 4.35, 2.89, and 12.65 mg/mL, respectively, with PLP3 exhibiting stronger DPPH scavenging activity. However, the DPPH scavenging ability of PLP3 was lower than that of ascorbic acid at all the investigated concentrations.

3.8 •OH scavenging activity

The •OH is the most reactive free radical among the oxygen radicals. It severely attacks all the biological molecules by setting off free radical chain reactions (Wu et al., 2013). Figure 4C shows that the •OH scavenging abilities of purified perilla polysaccharides and ascorbic acid were concentration-dependent. The maximum •OH scavenging activities of PLP1, PLP2, PLP3, PLP4, and ascorbic acid were 78.51%, 92.99%, 93.67%, 42.33%, and 96.86% at a concentration of 5.0 mg/mL, respectively. The IC$_{50}$ values calculated from the regression equation for PLP1, PLP2, PLP3, and PLP4, and ascorbic acid were 3.05, 1.58, 0.99, 7.58, and 0.52 mg/mL, respectively.

3.9 ABTS scavenging activity

The ABTS scavenging activity is also commonly used to determine the antioxidant activity of a compound. Figure 4D shows the ABTS scavenging activities of PLP1, PLP2, PLP3, and PLP4, and comparison with that of ascorbic acid. The ABTS scavenging activities of all the four polysaccharide fractions increased with their increasing concentrations from 0.1 to 5.0 mg/mL. The maximum ABTS scavenging activities of PLP1, PLP2, PLP3, PLP4, and ascorbic acid were 78.95%, 94.88%, 43.63%, and 98.86% at a concentration of 5.0 mg/mL, respectively. The IC$_{50}$ values calculated from the regression equation for PLP1, PLP2, PLP3, and PLP4 were 2.3, 1.44, 0.95, and 5.69 mg/mL, respectively. Although PLP3 exhibited the highest ABTS scavenging activity among the purified polysaccharide fractions (followed by PLP2, PLP1, and PLP4), its scavenging activity was less than that of ascorbic acid.
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4 Conclusion

In this study, an RSM experimental design was successfully applied to isolate perilla polysaccharides using UAEE. The optimal conditions for polysaccharide extraction were as follows: a liquid-to-solid ratio of 41:1, an enzymatic time of 40 min, an enzymatic temperature of 49 °C, and an ultrasonic power of 204 W. Under the optimal extraction conditions, the experimental polysaccharides yield was 3.84% (n = 3), which was correlated with the value of 3.9% predicted by RSM model. Thus, the present study developed an efficient method for the extraction of water-soluble polysaccharides from perilla leaves. After purification of the extracted crude polysaccharides by DEAE-cellulose-52 and Sephadex G-100 chromatography, homogenous polysaccharide fractions, PLP1, PLP2, PLP3, and PLP4 were obtained. Analysis of the antioxidant activities of the polysaccharide fractions in vitro demonstrated that all the fractions had effective antioxidant activities, with PLP3 exhibiting the highest antioxidant activity. With the development of plant antioxidant research, more and more cosmetics, health products, food and drugs related to antioxidant active ingredients in plants have shown increasingly broad prospects for development (Chen & Huang, 2019). The experimental results showed that the antioxidant activity of polysaccharides from perilla leaves were relatively high in reducing power, DPPH, •OH and ABTS free radical scavenging system, and had a concentration-dependent manner. These findings suggest that polysaccharides extracted from perilla leaves could have potential applications as antioxidants in medical and food industries, and further characterization, in vivo antioxidant activities assays, and analysis of the antioxidant mechanism of perilla polysaccharides are necessary.

Figure 4. Antioxidant activities of PLP1, PLP2, PLP3, PLP4, and ascorbic acid: (A) reducing power, (B) DPPH radical scavenging activities, (C) OH scavenging activities, and (D) ABTS radical scavenging activities.
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