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Cellulase-assisted extraction and antioxidant activity of polysaccharides from *Rhizoma imperata*

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ABSTRACT

In this study, the cellulase-assisted extraction and antioxidant activity of the polysaccharides from *Rhizoma imperata* were investigated. To improve the yield of *R. Imperata* polysaccharides (RPs), the extraction conditions were optimized as follows: time, 69.48 min; temperature, 45.36 °C; pH, 4.58; cellulase amount, 1200 U/g. Under these optimum conditions, the yield of RPs reached 0.67% (w/w), and was higher than that of the traditionally aqueous extraction method. The sugar content in the RPs product reached up to 93.25% (w/w). The RPs product has high antioxidant activity including hydroxyl radical scavenging activity and 2,2-diphenyl-β-picrylhydrazyl radical scavenging activity at the concentration of 100 mg/mL.

1. Introduction

Imperata cylindrica (cogongrass) is a common perennial grass in many warm and temperate parts of Asia as well as Australia and South Africa (Bijli, Singh, Sridhara, Gaur, & Arora, 2002). *Rhizoma imperata* is a traditional Chinese herb. It has the functions of clearing heat, cooling blood, and hemostatic effect (Cui, Li, You, & Xu, 2012).

R. Imperata contains polysaccharides, triterpenes, lactones, organic acids, etc. The polysaccharides, the main component of *R. Imperata*, with the molecular weight of 8292 kDa, contain rhamnose, xylose, fructose, mannose and glucose with the molar ratio of 1:11.45:1.26:1.02:59.23 (Zhou, Zhang, Wang, & Wang, 2012). *R. Imperata* polysaccharides (RPs) had anti-tumor, antihypertensive, antibacterial, anti-inflammatory, hepatoprotective activities and enhanced immunity (Cui et al., 2012; Hansen, Vilsbøll, & Knop, 2010). However, the antioxidant activity of *R. Imperata* has not been reported frequently so far.

Maceration, mechanical rabbling, heat reflux, ultrasound assistance, and acidic hydrolysis are the common extraction methods of polysaccharides from plant tissues. However, these methods require either long extraction time, or high extraction temperature, or expensive equipments, or environmental pollution. In contrast, an enzyme hydrolytic technology seems environmentally safe and more effective in terms of polysaccharides yield (Qian, 2014). However, enzyme-assisted extraction of the RPs was also not frequently reported.

In this study, a cellulase-assisted extraction method for RPs was developed, the extraction conditions were optimized and the antioxidant activity of the RPs was investigated.

2. Materials and methods

2.1. Materials

R. Imperata was purchased from a local pharmacy in Xinpu, China. Cellulase, with an enzymatic activity of 30,000 U/g, was purchased from Beijing Shengshi Jiaming Technology Development Co. Ltd. (Beijing, China). Reagent-grade chemicals were used.

2.2. RPs extraction

RPs extraction was performed according to Qian (2014) with slight modifications. *R. Imperata* was dried in a hot air oven (JK-OOI-240A, China) at 60 °C for 2 h. The dried *R. Imperata* was then pulverized and sifted through a 60 mesh sieve to obtain a fine powder with approximately 8% moisture content (dry basis).

The *R. Imperata* powder was extracted with organic solvents (light petroleum, acetone and methanol) in a Soxhlet apparatus to separate liposoluble components, and then suspended in distilled water to yield a 1% (w/v) suspension in a reactor. The pH of the suspension was adjusted to 4.58, and 1200 U/g of cellulase was added. The reactor was maintained in a thermostatic water bath at 45.36 °C for 69.48 min.

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The extracts were filtered through a Whatman GF/A filter paper and concentrated to approximately 15% (w/v). The proteins were removed using the Sevag method (Li, Zhao, Zhou, & Wu, 2012), and the extracts were precipitated by adding five volumes of absolute ethanol, followed by filtration using a Whatman GF/A filter paper, and freeze drying. The % RPs yield was calculated using Eq. (1) as follows:

$$\text{Yield} = 100 \times \frac{W_2}{W_1} \quad (1)$$

where W_1 and W_2 represent the weights of the recovered RPs and the original dried *R. Imperata* powder, respectively.

2.3. Antioxidant activity assays

Hydroxyl radical scavenging activity (HRSA) of the hydrolysates was measured according to the method of Yao, Cao, and Wu (2013) with a slight modification. Hydroxyl scavenging activity of RPs was calculated as follows:

$$\text{HRSA}\ \ (\%) = \frac{A_1 - A_2}{A_1 - A_0} \times 100 \quad (2)$$

where A_0 is the absorbance of the reagent blank, A_1 is the positive control absorbance, and A_2 is the absorbance of the sample.

2-Diphenyl-β-picrylhydrazyl radical scavenging activity (DRSA) was measured by the method described by Yao et al. (2013). Briefly, 0.2 mL of DPPH free radicals (DPPH•) solution (400 μmol/L in dehydrated alcohol) was added to 1.0 mL of RPs solution, and then 2.0 mL of distilled water was added. The mixture was shaken and allowed to stand at room temperature in the dark for 30 min. The absorbance was measured at 517 nm against a blank (distilled water instead of RPs and DPPH• solution). Lower absorbance of the reaction mixture indicates higher free-radical scavenging activity. The scavenging percentage was calculated by the following equation:

$$\text{DRSA}\ \ (\%) = \frac{[A_0 - (A_1 - A_2)]}{A_0} \times 100 \quad (3)$$

where A_0 is the absorbance of the control (distilled water instead of RPs solution), A_1 is the absorbance of the sample and A_2 is the absorbance of the sample under identical conditions as A_1 with distilled water instead of DPPH• solution.

2.4. Analytical methods

The pH of the solution was recorded using a digital pH meter (Model PHS-3C; CD Instruments, China). Ash, moisture, total sugar and protein contents of the samples were determined according to standard methods (Hou, 2004).

2.5. Experimental design

A central composite design (CCD) was used to optimize the extraction conditions utilizing cellulase and for fitting a polynomial model as follows:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 \times X_2 + \beta_{13} X_1 \times X_3 + \beta_{23} X_2 \times X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 \quad (4)$$

where Y is the PD yield, β_0 is the intercept term, β_1, β_2 and β_3 are linear coefficients, β_{12}, β_{13} and β_{23} are interaction coefficients, β_{11}, β_{22} and β_{33} are squared coefficients and X_1, X_2 and X_3 are coded independent variables. Design Expert software (version 8.0.5.0, State-Ease Inc., Minneapolis, USA) was used for the experimental design, data analysis, and model building.

Table 1
The central-composite design for optimizing extraction conditions.

Run	X_1	X_2	X_3	Yield (%)
1	60.00	45.00	5.34	0.61
2	60.00	45.00	4.50	0.65
3	60.00	53.41	4.50	0.35
4	43.18	45.00	4.50	0.51
5	50.00	50.00	4.00	0.41
6	60.00	45.00	4.50	0.69
7	60.00	45.00	4.50	0.67
8	50.00	50.00	5.00	0.47
9	76.82	45.00	4.50	0.68
10	60.00	45.00	3.66	0.58
11	70.00	50.00	4.00	0.54
12	50.00	40.00	4.00	0.43
13	70.00	50.00	5.00	0.56
14	60.00	45.00	4.50	0.64
15	60.00	45.00	4.50	0.66
16	60.00	36.59	4.50	0.31
17	70.00	40.00	5.00	0.53
18	50.00	40.00	5.00	0.45
19	60.00	45.00	4.50	0.66
20	70.00	40.00	4.00	0.51

X_1 = time (min), X_2 = temperature (°C), X_3 = pH.

2.6. Statistical analysis

All data are presented as mean ± SD. Statistical analysis was performed using Statgraphics Centurion XV version 15.1.02. A multifactor ANOVA with posterior multiple range test was used for determining statistical significance.

3. Results and discussion

3.1. Effect of time, temperature and pH on RPs extraction

The experimental design along with the experimental yields is shown in Table 1, while the results of regression analysis are listed in Table 2. The results were analyzed by using ANOVA, and the regression model obtained was as follows:

$$Y = -11.95069 + 0.033383 \times X_1 + 0.41342 \times X_2 + 0.91767 \times X_3 + 1.50000E-004 \times X_1X_2 - 1.0000E-003 \times X_1X_3 + 2.0000E-003 \times X_2X_3 + 2.56307E-004 \times X_1^2 - 4.77290E-003 \times X_2^2 - 0.10252 \times X_3^2 \quad (5)$$

where Y is the RPs yield (%, w/w), X_1 is the time (min), X_2 is the temperature (°C) and X_3 is the pH. The results confirmed that the regression model were statistically significant, as demonstrated by the F and P values [$(P > F) < 0.0001$]. High R^2 value (96.82%) indicated that the proposed regression model could successfully predict the influence of the three variables on the yield of RPs. In general, a regression model with an R^2 value > 0.9 is considered to have very high correlation (Haaland, 1989).

The interactions between time, temperature, and pH were not significant (Table 2). In order to find out the maximum yield of RPs, the quadratic regression equation (5) was solved analytically by using Microsoft Excel Solver Add-in., and the optimum conditions of time, temperature and pH for obtaining maximum RPs yield were calculated as 69.48 min, 45.36 °C and 4.58, respectively. Under these optimized conditions, maximum RPs yield was predicted to be 0.67% (w/w). The maximum RPs yield obtained experimentally using the optimized conditions was 0.66% (w/w), which was consistent with the predicted value obtained using a response surface methodology regression analysis.

Table 2
Analysis of variance for the experimental results of the central-composite design.

Factor	Sum of square	Degree of freedom	*F* value	*P* > *F*	Significance
X_1	0.032	1	147.26	0.0001	**
X_2	1.186E−003	1	5.38	0.0428	*
X_3	2.127E−003	1	9.65	0.0111	*
X_1^2	9.467E−003	1	42.94	<0.0001	**
X_2^2	0.21	1	930.57	<0.0001	**
X_3^2	9.467E−003	1	42.94	<0.0001	**
$X_1 \times X_2$	4.500E−004	1	2.04	0.1836	
$X_1 \times X_3$	2.000E−004	1	0.91	0.3633	
$X_2 \times X_3$	2.000E−004	1	0.91	0.3633	
Model	0.25	9	124.41	<0.0001	**
Lack of fit	7.216E−004	5	0.49	0.7761	
Pure error	1.483E−003	5			

X_1 = time (min), X_2 = temperature (°C), X_3 = pH.
* Statistically significant at 95% of probability level.
** Statistically significant at 99% of probability level.

3.2. Effect of cellulase amount on RPs extraction

The amount of cellulose is important for the efficient hydrolysis of RPs and may also play an important role in governing the efficiency of RPs extraction. Maximum RPs yield was obtained at 1200 U/g of cellulase (Fig. 1). In contrast to our findings here, the traditionally aqueous extraction method was used to extract RPs by Wang, Wu, Shi, and Gao (2010), and the optimum extraction conditions were extraction times 3, temperature 85 °C, volumes of water 15, and extraction time 3 h. Under these optimum extraction conditions, they obtained the maximum RPs yield [0.15% (w/w)], which was lower than obtained here.

3.3. Product characterization

The ash, moisture, and total sugar contents in the RPs product were 1.3%, 4.1% and 93.25% (w/w), respectively. The RPs product samples were water-soluble and showed white.

3.4. HRSA and DRSA of RPs

Hydroxyl radical (HO•) has the highest activity among reactive oxygen species and induces severe damage to biomolecules, while DPPH• is one of stable free radicals. Both HO• and DPPH• have been widely used as a tool for the estimation of the free-radical scavenging activities of antioxidants (Qiao et al., 2009). Both HRSA and DRSA were presented in Fig. 2. The scavenging effects of RPs increased with concentration increasing up to 100 mg/mL.

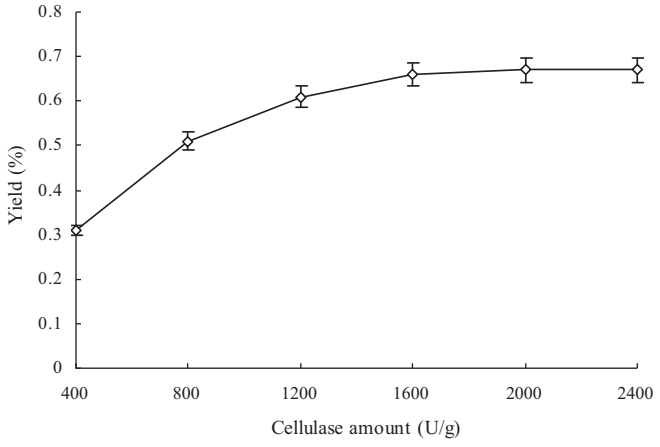

Fig. 1. Effect of cellulase amount on extraction of *Imperata cylindrica* polysaccharides. Bars represent the standard deviation. Data are shown as mean ± SD (n = 3).

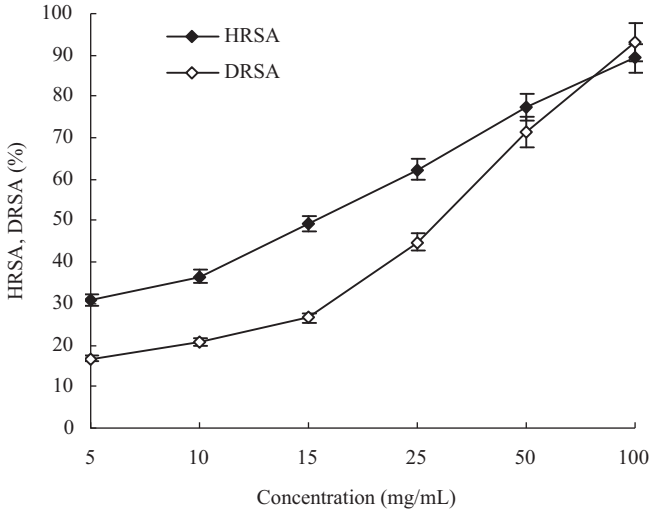

Fig. 2. Hydroxyl radical scavenging activity (HRSA) and 2,2-diphenyl-β-picrylhydrazyl radical scavenging activity (DRSA) of *Imperata cylindrica* polysaccharides. Data are shown as mean ± SD (n = 3).

At the concentration of 100 mg/mL, HRSA and DRSA were 89.23% and 93.24%, respectively, indicating that RPs possess high radical scavenging activity.

4. Conclusions

RPs could be effectively extracted by cellulase-assisted extraction method, and the yield of RPs was affected by extraction time, temperature, pH and the amount of cellulase. The sugar content in the RPs product was high as demonstrated by composition determination. The results show that RPs have high antioxidant activity and may be a viable option for use as a food antioxidant agent.

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