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Short communication

Cellulase-assisted extraction and antibacterial activity of polysaccharides from the dandelion *Taraxacum officinale*

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ABSTRACT

In the present study, we investigated the cellulase-assisted extraction and antibacterial activity of water-soluble polysaccharides from the dandelion *Taraxacum officinale*. The extraction conditions, optimized for improving yield, were as follows: time, 46.11 min; temperature, 54.87 °C; pH, 4.51 and cellulase enzyme, 4000 U/g. Under these conditions, the yield of polysaccharides from dandelion (PD) reached 20.67% (w/w). The sugar content of PD was 95.6% (w/w), and it displayed high antibacterial activity at a concentration of 100 mg/mL against *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus*. These results indicate that PD may be a viable option for use as a food preservative.

1. Introduction

Dandelion (*Taraxacum officinale*), a member of the Asteraceae/Compositae plant family, is a perennial herb native to the Northern hemisphere (You et al., 2010). Dandelion has been used as a phytomedicine for its choleretic, diuretic, anti-rheumatic, anti-diabetic and anti-inflammatory properties (Bisset, 1994; Schütz, Carle, & Sxhieber, 2006).

Extracts from different parts of the dandelion have been reported to exert various pharmacological effects. For instance, the aqueous extract from dandelion roots reduce alcohol-induced oxidative stress (You et al., 2010); dandelion leaf extract alleviated high-fat diet-induced non-alcoholic fatty liver (Davaatseren et al., 2013); and dandelion flower extract suppressed both reactive oxygen species and nitric oxide and prevented lipid oxidation in vitro (Hu & Kitts, 2005). Moreover, peptides with unusual cysteine motifs found in the flowers of *T. officinale* were reported to display antimicrobial activity (Astafieva et al., 2012). However, insufficient data are available regarding the possible functions of the polysaccharides in dandelion (PD).

In this study, a cellulase-assisted extraction procedure for PD was developed, the extraction conditions were optimized using a response surface design and the antibacterial activity of PD was investigated.

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2. Materials and methods

2.1. Materials

Dandelion shoots were 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. PD extraction

Dandelions were dried in a hot air oven (JK-OOI-240A, China) at 45 °C until a constant weight was observed. The dried dandelions were then pulverized and sifted through a 60 mesh sieve to obtain a fine powder with approximately 9% moisture content (dry basis), which was stored in dark bags in a dry environment until use.

The dried dandelion powder was extracted with organic solvents (light petroleum, acetone and methanol) in a Soxhlet apparatus, and then soaked in distilled water to yield a 1% (w/v) suspension. The pH of the suspension was adjusted to 4.51, and 4000 U/g of cellulase was added. The reactor was maintained in a thermostatic water bath at 54.87 °C for 46.11 min.

The extract was filtered through a Whatman GF/A filter paper and concentrated to approximately 20% (w/v). The proteins were removed using the Sevag method, and the extract was precipitated by adding 3 volumes of absolute ethanol, followed by filtration using a Whatman GF/A filter paper, and freeze drying. The % PD yield was calculated using Eq. (1) as follows:

$$\text{Yield} = \frac{100W_2}{W_1} \tag{1}$$

where W_1 and W_2 represent the weights of the recovered PD and the original dried dandelion powder, respectively.

2.3. 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).

2.4. Antibacterial activity assay

Antibacterial activity assay was performed according to the method described by Qian (2014), with a slight modification. Common pathogens such as *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* were used for testing the antibacterial activity of PD. The test strains were subcultured, incubated for 1 h at 37 °C, and swabbed on to the surface of sterile, pre-solidified Mueller–Hinton agar plates. Sterile paper discs (8 mm in diameter) were placed on the petri dishes, and 100 mg/mL PD was added to each disc. Disc to which sterile water was added was used as an experimental control. The tests for assessing the antibacterial activity of PD were performed in duplicate, and the average values were reported. The presence of a zone of inhibition around the disc (8 mm diameter) indicates the sensitivity of the test bacteria to the PD sample. The total diameter of the zone of inhibition was measured for each test bacterium.

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:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 \times X_2 + \beta_{13} X_1 \times X_3 \times X_3 + \beta_{11} X_{12} + \beta_{22} X_2^2 + \beta_{33} X_3^2 \tag{2}$$

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 7.1.6, State-Ease Inc., Minneapolis, USA) was used for the experimental design, data analysis and model building.

2.6. Statistical analysis

All data are presented as mean ± S.D. 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 PD extraction

The optimum combination of the three variables, i.e., time, temperature and pH, was investigated using a CCD. The design and results of the experiments conducted using CCD are shown in Table 1. The results were analysed using ANOVA, and the regression model was obtained as follows:

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

Run	X_1	X_2	X_3	Yield (%)
1	40.00	55.00	5.34	7.69
2	40.00	63.41	4.50	6.34
3	50.00	60.00	4.00	13.39
4	30.00	50.00	4.00	5.82
5	40.00	50.00	3.66	12.36
6	30.00	50.00	5.00	9.34
7	50.00	50.00	5.00	14.59
8	40.00	55.00	4.50	22.36
9	30.00	60.00	5.00	9.87
10	30.00	60.00	4.00	11.36
11	56.82	55.00	4.50	20.58
12	40.00	55.00	4.50	19.57
13	40.00	55.00	4.50	20.32
14	40.00	55.00	4.50	19.91
15	50.00	60.00	5.00	11.25
16	40.00	55.00	4.50	19.81
17	50.00	50.00	4.00	12.41
18	40.00	55.00	4.50	20.56
19	23.18	55.00	4.50	15.34
20	40.00	46.59	4.50	6.23

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

$$Y = -1067.35549 + 2.23414 \times X_1 + 24.87355 \times X_2 + 158.16926 \times X_3 - 0.021075 \times X_1X_2 - 0.049750 \times X_1X_3 - 0.46650 \times X_2X_3 - 8.43687E-003 \times X_1^2 - 0.19886 \times X_2^2 - 14.59653 \times X_3^2 \tag{3}$$

where Y is the PD yield (%, w/w), X_1 is the time (min), X_2 is the temperature (°C) and X_3 is the pH. The statistical significance of Eq. (3) was verified through ANOVA for the response surface quadratic model. The results (Table 2) indicate that the model was highly significant, as demonstrated by the F and P values [$(P > F) < 0.0001$]. The accuracy of fit was proven by the high value of multiple correlation coefficient (R^2 = 97.38%), indicating that the response model could explain 97.38% of the total variations. In general, a regression model with an R^2 value >0.9 is considered to have very high correlation (Haaland, 1989). The value of the adjusted multiple correlation coefficient (R^2_{Adj} = 95.02%) was also sufficiently high to indicate the statistical significance of the model.

The interaction between time and temperature and that between time and pH were significant, unlike that between temperature and pH (Table 2). On the basis of the model, the optimum conditions of time, temperature and pH for obtaining maximum PD yield were calculated as 46.11 min, 54.87 °C and 4.51, respectively. By substituting values of the factors into the regression equation, the maximum predictable response for the PD yield was calculated and experimentally verified. The maximum PD yield obtained

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	42.40	1	27.77	0.0004	*
X_2	1.11	1	0.73	0.4136	
X_3	2.45	1	1.60	0.2340	
X_1^2	10.26	1	6.72	0.0269	*
X_2^2	356.18	1	233.30	<0.0001	**
X_3^2	191.90	1	125.70	<0.0001	**
$X_1 \times X_2$	8.88	1	5.82	0.0365	*
$X_1 \times X_3$	0.50	1	0.32	0.5816	
$X_2 \times X_3$	10.88	1	7.13	0.0235	*
Model	567.38	9	41.29	<0.0001	**
Lack of fit	10.12	5	1.97	0.2380	
Pure error	5.15	5			

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

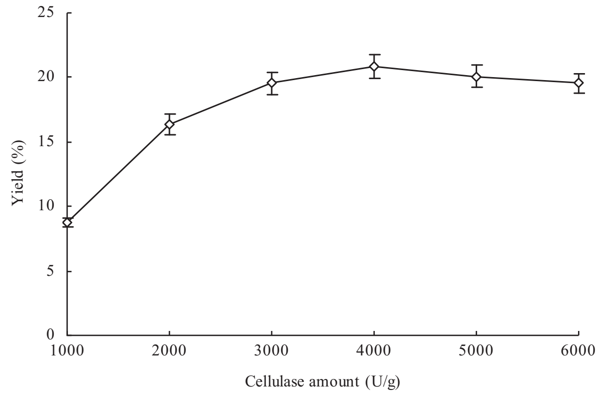

Fig. 1. Effect of cellulase amount on extraction of dandelion polysaccharides. Bars represent the standard deviation. Data are shown as mean ± SD (n = 3).

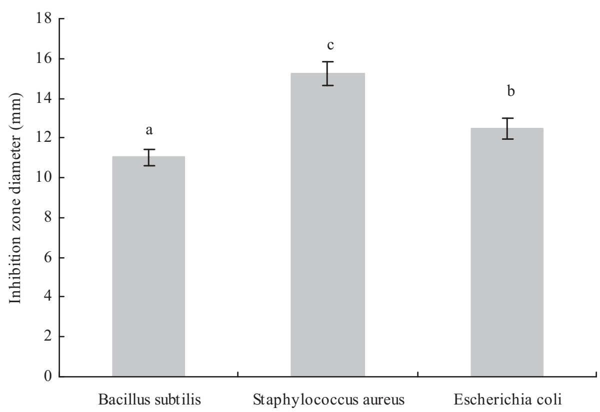

Fig. 2. Antibacterial activity of dandelion polysaccharides. Bars represent the standard deviation. Different letters on the bars indicate the significant differences ($P < 0.05$). Data are shown as mean ± SD (n = 3).

experimentally using the optimized conditions was 20.67% (w/w), which was consistent with the predicted value of 20.83% (w/w) that was obtained using a response surface methodology regression analysis.

3.2. Effect of cellulase amount on PD extraction

The amount of cellulase enzyme may also play an important role in governing the efficiency of PD extraction, which was investigated next. Optimum reaction conditions were obtained using 4000 U/g of cellulase (Fig. 1), in contrast to a previous study, in which optimum conditions for cellulase-assisted extraction of polysaccharides included a pH of 5.0, temperature of 56 °C, 3500 U/g cellulase and an extraction time of 40 min. These differences in the reported optimum pH, temperature, cellulase amount and extraction time could be due to differences in substrates and enzyme sources.

3.3. Product characterization

The ash, moisture and total sugar contents of the product samples were 1.1%, 1.6% and 95.6% (w/w), respectively. The product samples were water-soluble and did not contain any protein, which was consistent with the results of a previous study (Gao, Sun, Teng, and Hou, 2010).

3.4. Antibacterial activity of PD

The results of the antibacterial activity assay using PD are shown in Fig. 2. The diameter of the zone of inhibition formed by PD was found to be 11.02 mm, 15.26 mm and 12.47 mm for *B. subtilis*, *S. aureus* and *E. coli*, respectively, indicating a high antibacterial activity of PD. These results agree with those of a previous study (Song, Liu, Zhang, & Liu, 2010). Those authors observed that PD could inhibit the growth of *E. coli*, *S. aureus*, *Staphylococcus epidermidis*, *Salmonella typhimurium* and bacteria belonging to the genus *Streptococcus*.

4. Conclusions

In the present study, cellulase-assisted extraction of water-soluble PD was performed. The PD yield was affected by extraction time, temperature, pH and the amount of cellulase enzyme. Partial characterization of PD was performed, and its antibacterial activity against *B. subtilis*, *S. aureus* and *E. coli* was determined. Our results show that PD has high antibacterial activity and may be a viable option for use as a food preservative.

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