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Ultrasound assisted extraction of polysaccharides from *Lentinus edodes* and its anti-hepatitis B activity *in vitro*

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ABSTRACT

The aim of this study was to optimize the extraction process of polysaccharides from the fruiting bodies of *Lentinus edodes* and investigate its anti-hepatitis B virus activity. The extracting parameters including ultrasonic power (240–320 W), extraction temperature (40–60 °C) and extraction time (15–25 min) was optimized by using three-variable-three-level Box-Behnken design based on the single-factor experiments. Data analysis results showed that the optimal conditions for extracting LEPs were an extraction temperature of 45 °C, extraction time of 21 min and ultrasonic power of 290 W. Under these optimal conditions, the experimental yield of LEPs was 9.75%, a 1.62-fold increase compared with conventional heat water extraction (HWE). In addition, crude polysaccharides were purified to obtain two fractions (LEP-1 and LEP-2). Chemical analysis showed that these components were rich in glucose, arabinose and mannose. Furthermore, HepG2.2.15 cells were used as *in vitro* models to evaluate their anti-hepatitis B virus (HBV) activity. The results suggest that LEPs possesses potent anti-HBV activity *in vitro*.

1. Introduction

Lentinus edodes (Chinese name Xiang-Gu), a member of the Omphalotaceae family, is an edible-medicinal mushroom native to East Asian countries, in particular China. *L. edodes* is well known for its delicious taste and its rich nutrition. In recent years, a great deal of attention has been paid to *L. edodes* polysaccharides (LEPs) for its multiple beneficial bioactivities, including antioxidant [1], antitumor [2], immunosuppressive [3], and immunomodulating activities [4,5].

The conventional extraction methods for polysaccharides from plant samples were related to heating or boiling [6]. Despite their widespread use, these techniques have a tendency to achieve lower extraction yield and usually require abundant solvent, longer extraction times or higher extraction temperature. When solvent extraction is performed over a long period and at high temperatures, the structure of the polysaccharide may be destroyed and thus bioactivities declined or disappear completely. Recently, ultrasound-assisted extraction (UAE), which is considered to be a moderate, efficient, energy-saving and eco-friendly method, has been widely applied in the extraction procedure to improve the yield of target compounds from different plants [7–13].

Response surface methodology (RSM) is an effective statistical method for developing and optimizing complex processes in situations where the independent variables have a combined effect [14]. The main advantage of RSM is the dramatic reduction in the number of experimental trials. RSM has been successfully employed in food and pharmaceutical research [15–17].

In the present study, in order to enhance the yield of LEPs, UAE coupled with Box-Behnken design (BBD) was used to optimize and investigate process variables including extraction temperature, extraction time and ultrasonic power. In addition, DEAE-52 anion exchange chromatography and Sephadex-100 chromatography were utilized to separate crude polysaccharides to obtain two polysaccharide fractions and their chemical composition was evaluated. Finally, the anti-HBV activity of these fractions was investigated. This study may promote the exploration and development of potential values of polysaccharides from *L. edodes*.

2. Materials and methods

2.1. Materials and equipments

L. edodes was purchased from a supermarket in Zhangjiakou, China. D-Glucose was obtained from Adamas (Shanghai, China).

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Table 1
Coded and levels of independent variables and the response.

run	Extraction temperature (X_1)	Extraction time (X_2)	Ultrasonic power (X_3)	Yield (%)
1	1(60)	0(20)	1(320)	8.83
2	0(50)	1(25)	1(320)	7.56
3	0(50)	1(25)	−1(240)	4.36
4	0(50)	0(20)	0(280)	9.63
5	1(60)	1(25)	0(280)	6.37
6	−1(40)	−1(15)	0(280)	6.09
7	−1(40)	0(20)	−1(240)	6.31
8	0(50)	−1(15)	−1(240)	4.25
9	−1(40)	0(20)	1(320)	8.13
10	−1(40)	1(25)	0(280)	8.53
11	0(50)	0(20)	0(280)	9.79
12	1(60)	−1(15)	0(280)	6.68
13	0(50)	0(20)	0(280)	9.46
14	0(50)	−1(15)	1(320)	4.57
15	1(60)	0(20)	−1(240)	6.08

Lamivudine was obtained from GlaxoSmithKline (Fujian, China) and used as positive control. All other chemical reagents used in this study were of analytical grade. An ultrasonic generator was used for extraction (KQ-400KDE, Kunshan Ultrasonic Instruments C., LTD, Jiangsu, China).

2.2. Extraction procedures

2.2.1. Ultrasound-assisted extraction

In order to remove lipids from samples, the dried fruiting body of *L. edodes* was pulverized and defatted twice with petroleum ether (30–60 °C) for 12 h in a Soxhlet apparatus. The pretreated samples were separated and dried to constant weight at 30 °C in a vacuum oven. UAE was performed by using water as extraction solvent with an ultrasonic device at the given temperature, extraction time and ultrasonic power. The ultrasonic device was equipped with a temperature control panel and time regulator. The extract solution was centrifuged, filtered and concentrated. Subsequently, the crude extract was mixed with 4 vols of ethanol and stored at 4 °C overnight. After centrifugation for 15 min at 5000 rpm, the collected precipitates were dissolved in distilled water, deproteinized and lyophilized to obtain crude LEPs. The polysaccharides extraction yield (%) was calculated as follows:

$$\text{yield}\left(\%, \text{w/w}\right) = \frac{\text{Dried crude polysaccharides weight (g)}}{\text{Powder weight (g)}} \times 100\% \quad (1)$$

2.2.2. Hot water extraction (HWE)

According to reference [18], twenty grams of pretreated powder was mixed with 100 ml of distilled water and boiled for 8 h using a heating jacket. The mixture was centrifuged, filtered, concentrated, ethanol precipitated, deproteinized and lyophilized to obtain crude LEPs.

2.3. Experimental designs

RSM was applied to the experimental data in order to estimate the effect of extraction temperature, extraction time and ultrasonic power on the extraction yield of LEPs (%). Based on the preliminary range of the process variables in a single factor test, a BBD was performed with three independent variables at three levels. Fifteen experimental points were specified at random in order to reduce the effect on the observed responses. The range of independent variables and the actual levels of each factor is displayed in Table 1. The yield of LEPs (Y) was treated as the response for the interaction of the three variables. The data from BBD was described by the following nonlinear quadratic model:

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

Where Y is the yield of LEPs; X_1, X_2 and X_3 are independent variables representing extraction temperature, extraction time and ultrasonic power, respectively; β_0 is the intercept term; β_1, β_2 and β_3 are the regression coefficients corresponding to the independent variables; and β_{12}, β_{13} and β_{23} are the interaction coefficients corresponding to the independent variables; β_{11}, β_{22}, and β_{33} are the quadratic coefficients.

2.4. Preparation of polysaccharide fractions

The pretreated powder was extracted under the above optimal conditions, and the crude polysaccharides extract were purified sequentially by DEAE-52 anion-exchange chromatography and Sephadex G-100 chromatography according to the reported methods [19,20]. Briefly, 5 ml of polysaccharide solution (4 g/ml) was applied to the DEAE-52 column (2.6 × 30 cm), then stepwise elution with NaCl solutions (0-0.5 M) at a flow rate of 1.0 ml/min. Two collected fractions were concentrated, dialyzed and purified with deioned water through a Sephadex G-100 gel column. The two purified fractions, named LEP-1 and LEP-2, were collected, concentrated, dialyzed and lyophilized for further study.

2.5. General methods

The sugar content was measured at 490 nm by the phenol-sulfuric acid method with D-glucose as a standard [21]. Protein of the samples were evaluated according to standard methods [22]. The average molecular weight of LEPs was determined by gel permeation chromatography (GPC) method, and the molecular mass was measured by comparison with a series of known standard. The content of uronic acid was analyzed by the reference method [23] using glucuronic acid as the standard. The monosaccharide compositions were established by gas chromatography (GC) according to the previous reference [24]. The monosaccharide, including glucose, xylose, galactose, arabinose, mannose and rhamnose were used as standards.

2.6. Treatment of HepG2.2.15 cells with polysaccharides

HepG2.2.15 cells were seeded at a density of 8×10^4 cells/ml in 96-well plates and maintained at 37 °C for 24 h, and then treated with various concentrations of polysaccharides solution. After 6 days of treatment, the supernatants were collected inde-