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Optimization extraction and bioactivities of polysaccharide from wild *Russula griseocarnosa*

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ABSTRACT

The extraction conditions and biological activities of polysaccharides from wild Russula griseocarnosa (PRG) were investigated. Response Surface Methodology (RSM) with a Box-Behnken Design (BBD) was used to optimize extraction conditions. The optimal extraction parameters of PRG were as follows: extracting time 4 h, extraction temperature 77.3 °C and liquid-solid ratio 42.5 g/L. Furthermore, the data demonstrated that PRG exhibited antioxidant activities evidenced by reducing power to scavenge the DPPH, ABTS, hydroxyl radical and superoxide radical. PRG showed the activity of anti-cervical carcinoma cells Hela and Siha. In conclusion this study offered an efficient extraction method of wild Russula griseocarnosa polysaccharide, and the results suggested PRG had good antioxidant and inhibitory activities against cervical carcinoma cells, and PRG could be developed as a novel natural functional food.

1. Introduction

Polysaccharides are widely distributed in animal, plants and microorganisms, which is a large natural resource (Tao et al., 2016; Ma et al., 2013; Atta et al., 2017). Recently, considerable interest has arisen in characterizing the polysaccharide of mushrooms, which show various pharmacological functions, including anti-tumor, immunoregulatory and antioxidant activities (Du et al., 2016; Palanisamy et al., 2016; Tian et al., 2016; Sun et al., 2013). Polysaccharides from *Agrocybe cylindracea* SL-02 had enhancement effects on the reducing power of reactive oxygen species (Liu et al., 2016b). Additionally, polysaccharides from edible mushroom *Agaricus bisporus* (*Lange*) *Sing.Chaidm* was effective in scavenging ability on DPPH and hydroxyl radicals (Liu et al., 2016a; Li et al., 2015). Many studies have focused on the anti-tumor activities of polysaccharide. A neutral polysaccharide from *Letinus giganteus* had anti-proliferation of HepG2 cells via intrinsic mitochondrial apoptosis and PI3K/Akt signaling pathway (Chen et al., 2016; Tian et al., 2016; Halim et al., 2017; Muhammad et al., 2017).

It is wildely recognized that natural mushroom polysaccharides has tremendous potential for promoting human health. Efficient extraction techniques of polysaccharide are important for enlarging their application in the practical production. RSM was recognized a useful method combined with empirical and statistical characterization, which was widely employed in the commercial application for extraction conditions optimization in the process. It is an efficient method for improving and optimizing industrial processes (Sarfraz et al., 2016; Zheng et al., 2016). As known, the main advantages of RSM are to evaluate multiple factors and the interactions through less experimental trials (Wu et al., 2013; Ishaq and Jafri, 2017).

Russula griseocarnosa was originally discovered as a new species in China in 2009. The reports of bioactivity on *R. Griseocarnosa* remain less. In order to explore and use the polysaccharide from wild *R.griseocarnosa*, RSM combined with a BBD was employed to obtain the optimal polysaccharide extraction parameters in this paper. Then, the antioxidant and anti-tumor activities of PRG were studied.

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2. Materials and methods

2.1. The chemical reagents

The dry *R. griseocarnosa* sporocarp was purchased from Fujian, Southeast of China. The material was identified by Professor Bau Tolgor of Jilin agriculture university, Changchun, China.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) and 2,2-azino-bis (3-et hylbenzothiazoline-6-sulfonic acid) (ABTS) were purchased from sigma Co. The Fetal bovine serum (FBS) and DMEM medium were purchased from Gibco, Biomycs was purchased from Biological Industries. CCK8 kit was purchased from TransGen Biotech. Other chemicals including anhydrous ethanol, petroleum ether, phenol, acetone, sulfuric acid, potassium persulfate, H_2O_2, Trolox, Vitamin C and salicylic acid-ethyl alcol were purchased locally and were of analytical grade.

2.2. Extraction of polysaccharide

Dry *R. griseocarnosa* sporocarp were firstly grinded into power, and filtered with 40 mesh sieved. The polysaccharides of *R. griseocarnosa* were extracted as reported method with minor modifications.

Briefly, the dry filtered power was pretreated with petroleum ether and 95% ethanol to remove colored materials and other low molecular chemicals. Then the power were dried and extracted under the conditions with designed factors and their levels. The crude polysaccharides solution was concentrated, and resuspended with a three volumes of anhydrous ethanol and incubated. After 12 h, the crude polysaccharide solution with anhydrous ethanol were centrifuged at 3000*g* for 20 min, then the pellets were kept and washed with absolute ethanol and acetone. Finally, the precipitates were dried.

Table 1
The three variables and levels in quadratic orthogonal rotation combination design.

Independent variables	Symbol	Levels		
		−1	0	1
Time (h)	X_1	2	3	4
Temperature (°C)	X_2	70	80	90
Liquid-solid ratio (g/L)	X_3	30	40	50

2.3. Single factor design for polysaccharide of R. Griseocarnosa sporocarp

The range of 3 designed variables was firstly confirmed by the Single-Factor Design. The three key factors consisted of extraction time (X_1, 1, 2, 3, 4 and 5 h), extraction temperature (X_2, 50, 60, 70, 80 and 90 °C) and liquid-solid ratio (X_3, 20, 30, 40, 50 and 60 g/L). One factor changed according to the design while the other two factors kept unchanged, and the polysaccharide of *R. Griseocarnosa* (PRG) sporocarp were extracted as described in Section 2.2.

2.4. Experimental design of RSM

RSM was applied to predict the optimal extraction conditions of PRG according to the data of single-factor experiment. Then the Design Expert Software (8.0.6) was applied to the experiment design, data analysis and model building. The Box-Behnken Design (BBD) was used to explore the optimal levels of the 3 factors (X_1, X_2 and X_3) as shown in Table 1. Every factor was designed into three levels, and coded as −1, 0, +1 for low, intermediate and high levels, respectively. For statistical calculation, the 3 variables were coded as follows:

$$xi = \frac{(Xi - X0)}{\Delta Xi}$$

In the equation, X_i is the real value of factor, X_0 is the real value of the X_i at the center point, ΔX_i is the step change value in X_i, x_i is the coded value of the factor, and $i = 1, 2, 3$.

This BBD comprising of 17 experimental runs was finished in a random order in Table 2. There were 12 factorial points and 5 axial points which were used to allow for estimation of a pure error sum of squares, the experimental data were fitted to the following quadratic polynomial model:

$$Y = \beta 0 + \sum_{i=1}^{3} \beta iXi + \sum_{i=1}^{3} \beta iiX_i^2 + \sum_{i=1}^{3}\sum_{j>1}^{3} \beta ijXiXj$$

Y denotes the response values, β_0, β_i, β_{ij} and $\beta_{i\ i}$ are the constant coefficient, the linear coefficient, the interaction coefficients and the squared coefficients, respectively. X_i and X_j are the coded independent variables, X_iX_j and X_i^2 represent the interaction and quadratic terms.

The result analysis was finished with Design Expert software (8.0.6) and ANOVA. The fitness of the polynomial model equation was expressed by the coefficient of determination R^2 and the

Table 2
Box-Behnken design and results for the yield.

Run	Coded variable levels			Experimental values (%)	Predicted values (%)
	X_1	X_2	X_3		
1	−1.00	0.00	−1.00	4.21	4.28
2	0.00	0.00	0.00	5.48	5.36
3	−1.00	0.00	1.00	4.27	4.27
4	0.00	0.00	0.00	5.16	5.36
5	0.00	1.00	1.00	3.95	4.08
6	1.00	1.00	0.00	4.54	4.48
7	0.00	1.00	−1.00	4.46	4.52
8	0.00	0.00	0.00	5.30	5.36
9	0.00	0.00	0.00	5.43	5.36
10	1.00	0.00	−1.00	4.41	4.41
11	0.00	−1.00	−1.00	3.44	3.31
12	0.00	−1.00	1.00	4.30	4.24
13	1.00	−1.00	0.00	4.86	4.99
14	−1.00	−1.00	0.00	3.51	3.57
15	1.00	0.00	1.00	5.00	4.93
16	−1.00	1.00	0.00	5.25	5.12
17	0.00	0.00	0.00	5.43	5.36