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Extraction optimization and biological properties of a polysaccharide isolated from *Gleoestereum incarnatum*

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ABSTRACT

Extraction was optimized of polysaccharides from *Gleoestereum incarnatum* (GIP). The three parameters, extraction temperature, extraction time and the ratio of water to raw material, were optimized using the Box–Behnken design. As a result, the optimal extraction conditions were: extraction temperature 87.5 °C, extraction time 1 h and the ratio of water to raw material of 39.7 mL/g, where the highest yield of polysaccharide (13.18%) was obtained. GIP-II was the main fraction purified form GIP. GIP-II was composed of galactose, glucose, xylose, and mannose, with glucose was the predominant monosaccharide. GIP-II exhibited strong scavenging activities against DPPH and hydroxyl radials *in vitro*, as well as a strong inhibitory effect on the growth of HepG2 cells. The overall findings indicated that GIP-II is worthy of further exploration for its potential applications in antitumor drugs or health foods.

1. Introduction

Mushrooms have been known to be an important source of nutritional diet and medicine for thousands of years. Extensive studies have revealed that many species of mushrooms are good for improving human health and preventing diseases (Yang et al., 2009). Polysaccharides isolated from mushrooms have been correlated with multiple pharmacological properties, such as antioxidant (Wang, Zhang, Wang, & Wang, 2013), immunomodulatory (Zhao, Kan, Li, & Chen, 2005), reducing blood lipid (Yu et al., 2013), antidiabetic (Yang, Hsu, Lin, Hsu, & Chen, 2012) and antitumor activities (Sun, Wang, & Zhou, 2012).

Gloeostereum incarnatum is a species of fungi in the family Cyphellaceae. This is a monotypic genus containing the single species *G. incarnatum*, an edible mushroom native to China. Polysaccharides from the mycelium of *G. incarnatum* possess a wide range of biological properties, such as antioxidant (Wang, Zhang, Liu, Zhang, & Hou, 2012b), immunomodulation (Weng, Weng, & Qiu, 2009), anti-inflammatory (Zhang & Li, 1999) and antibacterial activities (Zhang, Zhu, Mu, Liu, & Hou, 2012a).

To the best of our knowledge, there are no reports available in the literature regarding the optimization of extraction of polysaccharides from the fruiting bodies of *G. incarnatum* (GIP) using response surface methodology (RSM). It is therefore interesting and attractive to extract them and investigate their biological activities. In this study, the extraction parameters of GIP were optimized using a three-level, three variable Box–Behnken design (BBD). GIP was then purified and the antioxidant and antitumor activities of purified polysaccharide from *G. incarnatum* were evaluated *in vitro*.

2. Materials and methods

2.1. Materials

The fruiting body of *G. incarnatum* was obtained from the Institute of mushroom of Yutai, Shandong, China. Standard monosaccharides (L-fucose, L-rhamnose, D-mannose, D-xylose, D-glucose, D-galactose, Fructose and D-arabinose), 1,1-diphenyl-2-picrylhydrazyl (DPPH), trifluoroacetic acids (TFA), DEAE cellulose-52, Sephadex G-100 and dimethyl sulfoxide (DMSO) were all purchased from Sigma–Aldrich. Cancer cell line HepG2 (human

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2.2. Preparation of crude GIP

The fruiting body of *G. incarnatum* was dried at 60 °C in an oven for 24 h and then powdered for this study. Then, the powder was pretreated twice with 90% ethanol to remove oligosaccharides, some colored materials and small molecules. Finally, the organic solvent was volatilized to obtain a pretreated dry powder. The dried powder (5.0 g) was extracted in a HH-6 water bath (Guohua Wiring Company, Shanghai, China) with distilled water using designed parameters. The suspension was cent[redacted] the insoluble residue was treated ag[redacted] The supernatants were collected a[redacted] volume using a vacuum rotary eva[redacted] precipitated by

Table 1a
The Box–Behnken design matrix and the results for extraction yield of crude polysaccharides from *Gleoestereum incarnatum*.

Run numbers	*A*: Extraction temperature (°C)	*B*: Ratio of water to material(mL/g)	*C*: extraction time (h)	Response (%)	Predict response (%)
1	0(80)	−1(20)	−1(1)	8.98	8.69
2	−1(70)	0(30)	1(2)	12.24	9.96
3	−1(70)	1(40)	0(1.5)	10.53	10.63
4	1(90)	0(30)	1(2)	10.82	11.04
5	0(80)	0(30)	0(1.5)	12.14	12.1
6	0(80)	0(30)	0(1.5)	12.01	12.09
7	−1(70)	−1(20)	0(1.5)	8.18	8.7
8	−1(70)	0(30)	−1(1)	9.62	9.4
9	0(80)	1(40)	1(2)	10.72	11.01
10	0(80)	1(40)	1(2)	12.64	12.76
11	1(90)	−1(20)	0(1.5)	10.06	9.96
12	0(80)	0(30)	0(1.5)	12.08	12.09
13	0(80)	−1(20)	1(2)	11.76	11.64
14	0(80)	0(30)	0(1.5)	12.16	12.19
15	0(80)	0(30)	0(1.5)	12.09	12.1
16	1(90)	1(40)	0(1.5)	11.98	11.46
17	1(90)	0(30)	−1(1)	11.89	12.29

Table 1b
ANOVA for response surface quadratic model.

Variables	Sum of squares	DF	Mean square	*F* value	*P*-value
Model	26.05	9	2.89	17.14	0.0006
A	2.18	1	2.18	12.93	0.0088
B	5.93	1	5.93	35.13	0.0006
C	0.726	1	0.73	4.298	0.0768
AB	0.046	1	0.046	0.274	0.617
AC	3.4	1	3.404	20.153	0.0028
BC	5.525	1	5.522	32.696	0.0007
A^2	3.377	1	3.377	19.99	0.0029
B^2	4.321	1	4.32	25.581	0.0015
C^2	0.0142	1	0.014	0.0839	0.7805
Residual	1.1823	7	0.1689		
Lack of fit	1.1686	3	0.3895	113.5689	0.0003
Pure error	0.0137	4	0.00343		
Cor total	27.235	16			
R^2	0.957				
Adj R^2	0.9007				
Pred R^2	0.3127				
Adeq precision	12.92				
CV%	3.679				

3. Results and discussion

3.1. Fitting the process models

Based on preliminary single-factor experiments, the following conditions were adopted in the RSM experiments: extraction temperature, 70–90 °C; ratio of water to raw material, 20–40 and extraction time 1–2 h (data not shown).

As seen in Table 1a, results showed that the yield of polysaccharides ranged from 8.18 to 12.64%. Multiple regression analysis was performed on the experimental data and the relationship of the response variable and test variables was given by the following second-order polynomial equation:

$$Y\ (\text{yield}) = 12.10 + 0.52A + 0.86B + 0.30C - 0.11AB - 0.92AC - 0.12BC - 0.90A^2 - 1.01B^2 - 0.06C^2$$

Analysis of variance (ANOVA) results for the model are given in Table 1b. Extraction temperature and ratio of water to raw material exhibited significant impact on the extraction yield of GIP (*P*-values less than 0.05), while the effects of extraction time failed to reach statistical significance. The quadratic term of extraction temperature and ratio of water to raw material indicated that the two variables had a larger effect.

The model fits well with the experimental data, which was approved by the high values of determination coefficient R^2 (95.7%) and the adjusted determination coefficient Adj. R^2 (90.07%). The low coefficient value of the variation (CV = 3.679%) clearly suggested that a high degree of precision and a good deal of reliability of the experimental values. This result implied that polysaccharides extraction results could be analyzed and predicted by the model.

The effects of variables and their interactions on the yield of polysaccharides are illustrated by the 3D response surfaces and the 2D contour plots. Results from Fig. 1 further confirmed ANOVA findings. During GIP extraction, the temperature and water to solid ratio are more important. Therefore, the best combinations of process variables for response functions are obtained. Using Design-Expert, the optimum values for the tested variables for extraction of GIP were: an extraction temperature of 87.5 °C, an extraction time of 1 h and a ratio of water to raw material of 39.7 mL/g. the maximum predicted extraction yield of GIP was 13.26%, which corresponded well with the actual yield (13.18 ± 0.13%, $n = 3$). These results suggested that the model designed in this study was valid.

3.2. Purification of GIP

As shown in Fig. 2a, GIP was fractionated by DEAE-52 cellulose column chromatography to obtain three fractions. The second fraction was the main fraction, representing 65.25% of GIP. This fraction was further purified on a Sephadex G-100 column to obtained GIP-II. As shown in Fig. 2b, GIP-II yielded a single peak.

3.3. Preliminary characterization of GIP-II

HPAEC analysis showed that GIP-II was composed of galactose, glucose, xylose, and mannose at molar ratios of 1: 4.25: 1.14: 1.85. Glucose was the predominant monosaccharide (Fig. 3). The linearity of the method was calibrated using dextran standards of different molecular weights and the average Mw of GIP-II was about 42.87 kDa (data not shown).

3.4. Antioxidant activity of GIP-II

3.4.1. DPPH scavenging effect

The DPPH scavenging assay is widely used to test antioxidant activity because it is simple, rapid and sensitive (Benvenuti, Pellati, Melegari, & Bertelli, 2004). The observed DPPH scavenging activity of GIP-II is exhibited in Fig. 4a. The DPPH scavenging activity increased with increasing concentrations of GIP-II and BHA was stronger than that of GIP-II at every concentration point. At 0.8 mg/mL, the scavenging activities of GIP-II and BHA were 68.51

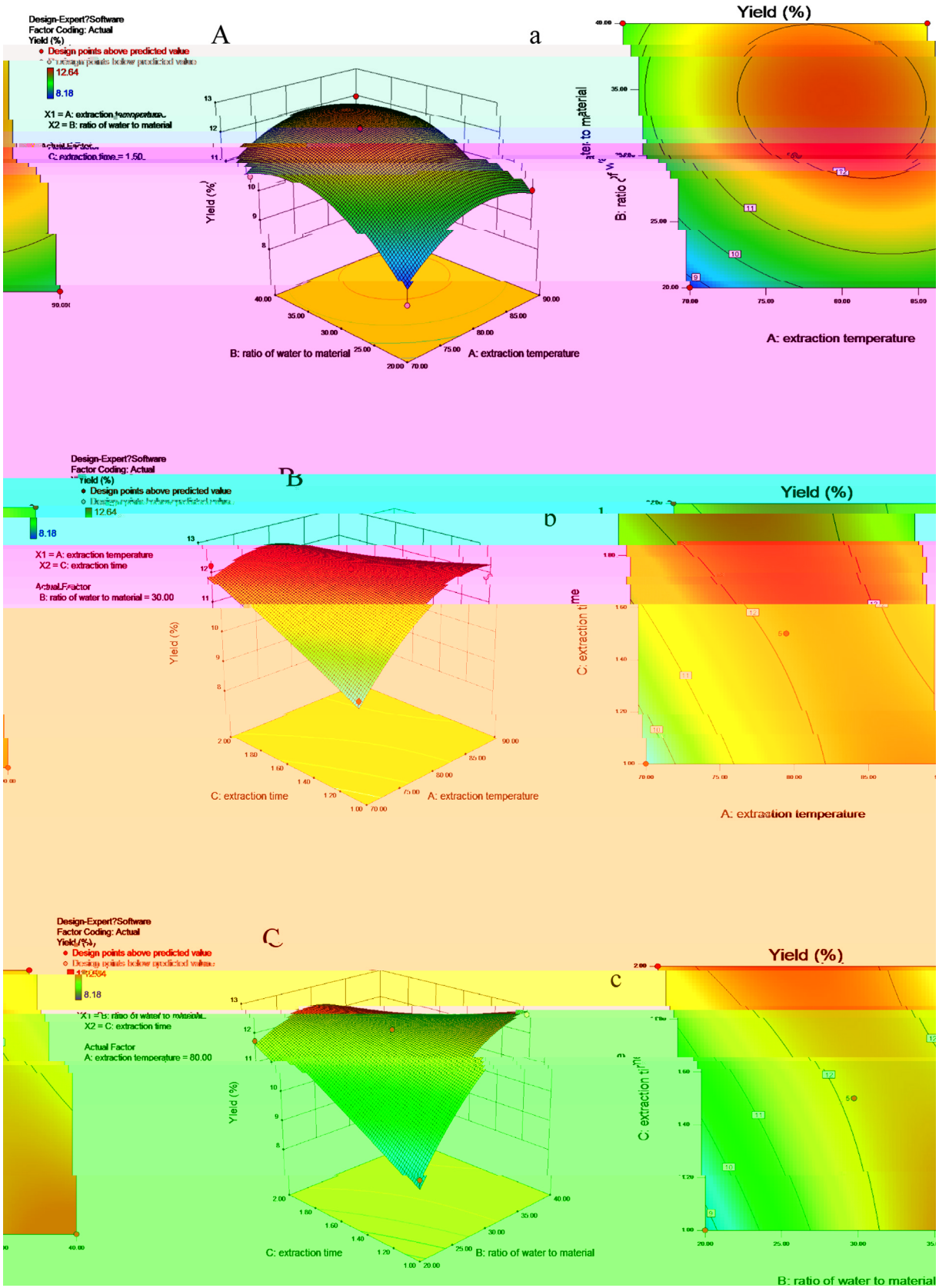
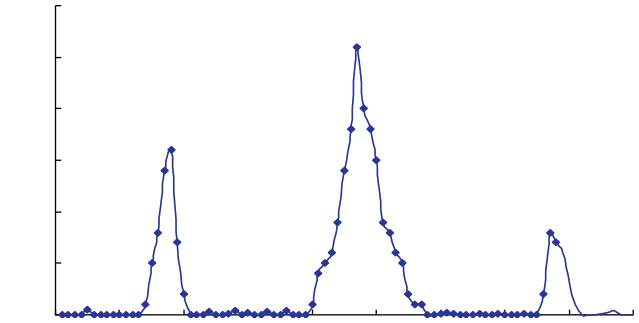
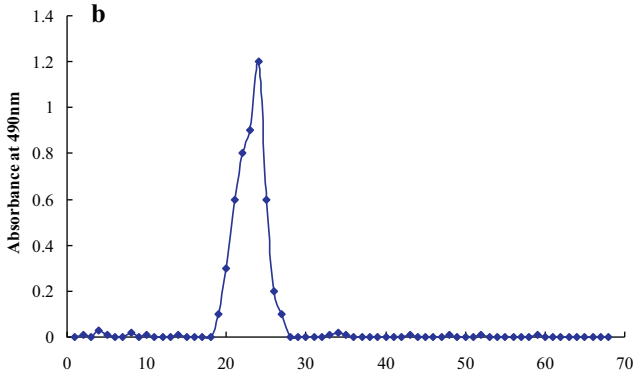


Fig. 1. 3D response surface plots (A–C) and 2D contour plots (a–c) showing the effects of temperature, time and ratio of water to materials on the yield of *Gleoestereum incarnatum* polysaccharide.

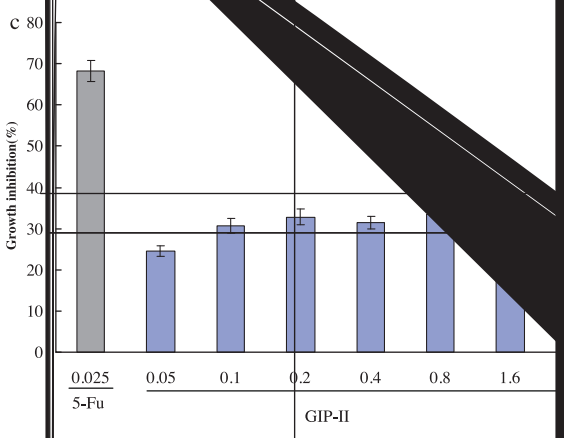



b
Absorbance at 490nm
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
10
20
30
40
50
60
70

a

Scavenging effect(%)

c



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