MATERIALE PLASTICE
https://revmaterialeplastice.ro
https://doi.org/10.37358/Mat.Plast.1964

Response Surface Optimization of the Alkaline Extraction Process of *Ganoderma lucidum* Polysaccharides and Preliminary Study on Their Immunological Activity

CHUANLI JIN, RUIAN MA, CHEN CHEN, ZE DANG, TONG YU, YU FU, JUAN DU*
Department of Pharmacognosy, College of Pharmacy, Jiamusi University, Jiamusi, 154007, China

Abstract: ***Objective:*** *To investigate and optimize the alkaline extraction process of Ganoderma lucidum polysaccharides, and to preliminarily explore their immunological activity.* ***Methods:*** *Ganoderma lucidum was used as the raw material for the extraction of polysaccharides. A single-factor experiment was conducted to examine the effects of NaOH concentration, temperature, and extraction time on the total sugar content of the polysaccharides. Based on these results, response surface methodology was applied to optimize the extraction process. The total polysaccharide content, uronic acid content, monosaccharide composition, molecular weight, and cell viability were measured.* ***Results:*** *The optimal extraction conditions were found to be a temperature of 93°C, NaOH concentration of 0.40 mol/L, and extraction time of 172 min, yielding a total polysaccharide content of 47.66%. The monosaccharide composition of the extracted polysaccharides included mannose, glucuronic acid, glucose, galactose, arabinose, and fucose. Molecular weight analysis revealed two average molecular weights, 3.15 × 10^4 Da and 1.014 × 10^4 Da, indicating the polysaccharides were relatively small. Infrared spectroscopy showed the presence of β-type glycosidic linkages in the polysaccharides. In the cell viability assay, GLCP-1 enhanced the viability of RAW264.7 cells and significantly inhibited the viability of HepG2 cells. However, the specific regulatory mechanism remains unclear.* ***Conclusion:*** *The study successfully optimized the alkaline extraction of Ganoderma lucidum polysaccharides and demonstrated their potential immunological activity, providing a foundation for the future exploration of their bioactivity and industrial production.*

Keywords: *Alkaline extraction, Ganoderma lucidum polysaccharides, response surface, immunological activity*

1. Introduction

Ganoderma lucidum is the dried fruiting body of the fungus *Ganoderma lucidum* (Leyss. ex Fr.) Karst. of the family Polyporaceae. Both *Ganoderma lucidum* (Leyss. ex Fr.) Karst. and *G. sinense* Zhao Xu et Zhang are included in the 2020 edition of the "Chinese Pharmacopoeia" [1]. *Ganoderma lucidum* is distributed in regions such as Heilongjiang, Jilin, and Liaoning.

Ganoderma lucidum is rich in various bioactive components, including polysaccharides, triterpenoids, amino acids, alkaloids, nucleosides, and trace elements [2,3]. Polysaccharides are high molecular weight carbohydrates formed by the dehydration condensation of multiple monosaccharides [4], with complex structural composition and diverse pharmacological effects, including antitumor [5], immunomodulatory [6], anti-inflammatory [7], hypoglycemic [8], and central nervous system regulatory activities, making them highly valuable in medicineIn recent years, various extraction techniques have been widely applied in polysaccharide extraction. For example, Do et al. [9] optimized ultrasound-assisted enzymatic extraction (UAE) using response surface methodology to extract polysaccharides from Vietnamese red *Ganoderma lucidum*. The study found that the polysaccharide content in the extract reached a maximum of 32.08 mg/g. Tian et al. [10] employed alkaline extraction to isolate

**email: dujuan@jmsu.edu.cn*

polysaccharides from *Grifola frondosa*, where the polysaccharide (GFP) and β-glucan contents were 91.61% and 60.57%, respectively, significantly higher than those obtained through water extraction. Additionally, characterization techniques such as gas chromatography (GC), nuclear magnetic resonance (NMR), and Fourier-transform infrared spectroscopy (FTIR) have provided more precise tools for polysaccharide structural analysis. GC, NMR, and FTIR analyses have revealed the complex glycosidic bond structures of lentinan [11,12]. Research on the bioactivity of polysaccharides has advanced significantly in recent years. Li et al. [13] discovered that *Lycium barbarum* polysaccharides (LBP) protect pancreatic β-cells by inhibiting the IFN-γ inflammatory pathway, improving glucose tolerance in diabetic mice. Wei et al. [14] used ultrasonic extraction to significantly enhance the yield and antioxidant capacity of *Cercis chinensis* polysaccharides, with uronic acid content positively correlated with bioactivity. Yan et al. [15] confirmed that optimized *Astragalus* polysaccharides delay aging by regulating amino acid metabolism pathways and reducing oxidative stress markers such as malondialdehyde (MDA). Studies on hypoglycemic activity reveal that yam polysaccharides not only inhibit α-glucosidase but also improve insulin resistance by modulating the AMPK signaling pathway [16]. These findings provide critical insights for the application of polysaccharides in functional foods and pharmaceutical development.

Gu et al. [17] and others found that alkaline-extracted *Ganoderma lucidum* polysaccharides exhibited superior immunological activity compared to water-extracted polysaccharides, and Huang et al. [18] found that alkaline treatment could break down fibers, accelerating the release of *Ganoderma lucidum* polysaccharides. These studies suggest that alkaline-extracted *Ganoderma lucidum* polysaccharides have great research potential.

Traditional extraction methods include water extraction, alkaline extraction, microwave extraction, and enzyme-assisted extraction [19]. Alkaline extraction is one of the commonly used methods for polysaccharide extraction. Compared to water extraction, alkaline extraction causes more significant disruption to cell wall structures, which facilitates polysaccharide solubilization [20]. The extraction of polysaccharides using complex enzymes has been reported [21], but the extraction cost is high and significantly impacts polysaccharide structure [22]. Microwave extraction of polysaccharides is also an advanced technique; however, microwaves generate heat through the friction of polar molecules, and localized high temperatures (e.g., $>80°C$) may disrupt glycosidic bonds in polysaccharides. This effect is particularly pronounced in acidic polysaccharides or low-molecular-weight polysaccharides, leading to reduced molecular weight and diminished bioactivities (such as antioxidant and immunomodulatory properties). Consequently, the alkaline extraction method was selected for polysaccharide extraction [23].

Currently, there are few studies on the alkaline extraction of *Ganoderma lucidum* polysaccharides and subsequent research. This experiment uses the Box-Behnken design to optimize the extraction process of *Ganoderma lucidum* polysaccharides, conducts preliminary structural analysis, and studies its immunological activity, aiming to provide a reference for the further development of *Ganoderma lucidum* polysaccharides.

2. Materials and methods

2.1. Materials and equipment

Ganoderma lucidum was purchased from the Jiamusi Traditional Chinese Medicine Hospital. Sodium hydroxide, phenol, anhydrous ethanol, sulfuric acid, etc., were all domestic analytical reagents. CCK-8 assay kits were purchased from Shanghai Beyotime Biotechnology Co., Ltd. (Shanghai, China) Various monosaccharide standards, such as mannose (Man), rhamnose (Rha), glucuronic acid (GlcA), galacturonic acid (GalA), glucose (Glc), galactose (Gal), xylose (Xyl), arabinose (Ara), and 1-phenyl-3-methyl-5-pyrazolone (PMP), were purchased from Sigma-Aldrich, St. Louis, MI, USA.

800Y high-speed multifunctional grinder (Wuyi Haina Electrical Appliance Co., Ltd. (Wuyi, China)); constant temperature water bath (JOANLAB Instruments Co., Ltd. (Huzhou, China)). BY101

electronic balance, Shanghai Chengyu E-commerce Co., Ltd. (Shanghai, China), PHS-3C microcomputer pH meter, Shanghai Yueping Electronic Instruments Co., Ltd. (Shanghai, China), 723N UV-Vis spectrophotometer, Shanghai INESA Analytical Instrument Co., Ltd. (Shanghai, China), 1260 high-performance liquid chromatography, Agilent Technologies, Santa Clara, CA, USA. Ultra-low temperature freezer, Zhongke Meiling Cryogenics Co., Ltd. (Hefei, China). RE-52A rotary evaporator, Shanghai Yarong Biochemical Instrument Factory (Shanghai, China). High-speed centrifuge, Changzhou Jintan Dadi Automation Instrument Factory (Changzhou, China). Vacuum freeze dryer, Shanghai Lichen Instrument Technology Co., Ltd. (Shanghai, China). Microplate reader, Tecan Group Ltd., Männedorf, Switzerland.

2.2. Experimental methods

2.2.1 Process flow

The *Ganoderma lucidum* powder was dried and ground, then extracted three times using 0.5 mol/L NaOH solution at a ratio of 1:20 at 80°C for 2 h per extraction. The supernatants were combined. The alkaline extraction supernatant was neutralized with 3 mol/L HCl, concentrated under vacuum at 40°C, and precipitated with four times the volume of 95% ethanol in a 4°C refrigerator to obtain crude polysaccharides. The precipitate was centrifuged at 8000 rpm for 10 min, redissolved in water, dialyzed, and freeze-dried to obtain alkaline-soluble crude polysaccharides (GLCP-1).

The extraction temperature, material-to-liquid ratio, and NaOH concentration were evaluated through single-factor experiments. Based on these conditions, a response surface experiment was conducted to clarify the optimal alkaline extraction process of *Ganoderma lucidum* polysaccharides.

2.2.2 Single-factor experiment

Precisely weighed 1.000 g of *Ganoderma lucidum* powder, set the base conditions as extraction temperature 80°C, extraction time 120 min, material-to-liquid ratio 1:20 g/mL, and NaOH concentration 0.3 mol/L. The levels of each factor were set as follows: extraction temperature 60, 70, 80, 90, 100°C, extraction time 60, 90, 120, 150, 180 min, and NaOH concentration 0.1, 0.2, 0.3, 0.4, 0.5 mol/L. The experiment was repeated three times to study the effect of different factors on the yield of alkaline-extracted *Ganoderma lucidum* polysaccharides.

2.2.3 Response surface experiment design

Based on the single-factor experiment, NaOH concentration (A), material-to-liquid ratio (B), and extraction temperature (C) were selected as independent variables, and the yield of *Ganoderma lucidum* polysaccharides was taken as the response value. The Box-Behnken design was used for a three-factor, three-level response surface experiment. The experimental factors and levels are shown in Table 1.

Table 1. Response surface factor level table

Levels	Factor		
	A-NaOH concentration mol/L	**B-Material-to-liquid ratio mol/L**	**C-Temperature mol/L**
−1	0.3	0.4	0.5
0	80	90	100
1	120	150	180

2.2.4 Total sugar content measurement

Take 2 mg of GLCP-1 powder, add 2 mL of water to prepare a 1 mg/mL solution. Using the anthrone-sulfuric acid method, measure absorbance at a wavelength of 490 nm, with three parallel groups.

Glucose is used as the standard to draw the standard curve, with concentration as the *x*-axis and absorbance as the *y*-axis. The total sugar content is calculated from this curve.

The calculation formula for the polysaccharide yield after alkali extraction of *Ganoderma lucidum*:

$$W = \frac{C \times V_1 \times \mathrm{n}}{M \times V_2}$$

In the formula: W: Total soluble polysaccharide content (mg/g); C: Sugar content derived from the standard equation (mg/g); V_1: Volume of extract (mL); V_2: Volume of sample solution taken (mL); M: Mass of the sample weighed (g); n: Dilution factor.

2.2.5 Measurement of uronic acid content in alkali-extracted Ganoderma lucidum polysaccharides

Determination of maximum absorption wavelength

Take 0.5 mL of 1 mg/mL glucuronic acid standard solution in a 10 mL volumetric flask, fill to volume with distilled water, and mix well. Transfer 1 mL to a stoppered test tube, add 6 mL of borax-sulfuric acid solution in an ice water bath, mix, and react for 5 min in a boiling water bath. Immediately cool in an ice water bath, then add 0.1 mL of m-hydroxybiphenyl solution (prepared by dissolving 150 mg of m-hydroxybiphenyl standard in 100 mL of 0.5% NaOH solution). After 5 min of color development, shake, remove bubbles by ultrasonication, and perform full-wavelength scanning. Prepare a 0.4 mg/mL GLCP-1 solution and conduct the same procedure for full-wavelength scanning.

Drawing of the glucuronic acid standard curve

Precisely take 0.35, 0.40, 0.45, 0.50, and 0.55 mL of glucuronic acid standard solution into 10 mL volumetric flasks, fill to volume with water, take 1.0 mL, and measure the absorbance (A) at the maximum absorption wavelength according to the method in "1.2.5.1." Draw the standard curve.

Measurement of uronic acid content

Take 1.0 mL of 0.4 mg/mL GLCP-1 sample solution, add 4.0 mL of water, mix well, and then take 1.0 mL. Measure the absorbance (A) at the maximum absorption wavelength, insert the A value into the regression equation, and calculate the uronic acid content in *Ganoderma lucidum*.

2.2.6 Measurement of monosaccharide composition

Preparation of standards

Accurately weigh 1.0 mg of each monosaccharide standard (Man, Rha, GlcA, GalA, Glc, Gal, Xyl, Ara, Fuc), mix thoroughly with distilled water, take 1 mL of the mixed standard solution, and add 0.6 mol/L NaOH solution and 0.5 mol/L PMP methanol solution. Mix well, react in a 70°C water bath, and after the reaction, neutralize with 0.3 mol/L HCl, extract with an equal volume of chloroform, filter through a microporous membrane, and transfer to a liquid-phase bottle.

Preparation of polysaccharide samples

According to the method of DAI [24], with slight modifications, weigh 1.0 mg of *Ganoderma lucidum* polysaccharides, add 1 mL of TFA solution, and hydrolyze for 2 h. After the acid evaporates and no acid smell remains, dissolve it in distilled water again. Add 0.6 mol/L NaOH solution and 0.5 mol/L PMP methanol solution, mix thoroughly, and react in a 70°C water bath. After the reaction is complete, neutralize with 0.3 mol/L HCl, then add an equal volume of chloroform solution for extraction. Filter using a microporous membrane, then transfer to a liquid chromatography vial.

2.2.7 Molecular weight determination

Following Yang Yi's method [25], with slight modifications, use HPGPC with 0.1 mol/L sodium nitrate solution as the mobile phase and pullulan standards to obtain the standard curve. Dissolve the polysaccharide samples and standards in the mobile phase at a concentration of 1 mg/mL, inject 50 µL, set the system temperature to 40°C, and flow rate to 0.5 mL/min.

2.2.8 FT-IR spectroscopy analysis

Mix 1 mg of the polysaccharide sample with 100 mg of KBr powder, compress into a pellet, and measure the characteristic peaks of the functional groups in *Ganoderma lucidum* polysaccharides. Infrared scanning is performed in the 4000–400 cm^{-1} range, with KBr powder as the background [26].

2.2.9 RAW 264.7 cell viability assay

Take logarithmic phase RAW 264.7 cells, and add media containing *Ganoderma lucidum* at final concentrations of 0, 8, 40, 100, 200, 500, 1000 μg/mL. Adjust cell density to 1×10^5 cells/mL with media, seed 100 μL of cell suspension per well in a 96-well plate, and culture at 37°C with 5% CO_2 for 3 days. Then, add 10 μL of 10% CCK-8 reagent to each well, incubate for 1.5 h, and measure the absorbance (OD) at 450 nm using a microplate reader [27].

Cell viability (%) = $(A_{sample} - A_0/A_{blank} - A_0) \times 100\%$

2.2.10 CCK-8 cell viability assay

Take logarithmic phase HepG2 cells, seed 100 μL per well into a 96-well plate at a density of 1×10^4 cells per well, and incubate the 96-well plate at 37°C with 5% CO_2. After 24 h, replace the culture medium with media containing *Ganoderma lucidum* polysaccharides at final concentrations of 0, 8, 40, 100, 200, 500, and 1000 μg/mL, and continue to culture in the incubator. After 48 h, add 10 μL of CCK-8 reagent to each well, incubate in the dark at 37°C in a 5% CO_2 incubator for 2 h, shake for 1 min, and measure the absorbance at 450 nm using a microplate reader [28].

3. Results and analysis

3.1. Single-factor experiment

Figure 1A shows that the polysaccharide content of alkali-extracted *Ganoderma lucidum* initially increases with increasing NaOH concentration, reaching a peak at 0.4 mol/L NaOH, and then decreases. This may be due to the fact that as NaOH concentration increases, the combination of alkali and high temperature leads to polysaccharide hydrolysis. The alkali breaks down the cell wall of *Ganoderma lucidum*, degrading the hydrolyzable bonds (such as O-linked side chains) between the cellulose structure and chitin and glucan, allowing more polysaccharides to dissolve. However, when the NaOH concentration becomes too high, the polysaccharides are hydrolyzed and their structure is destroyed, causing the polysaccharide content to decrease [29,30]. The highest polysaccharide content is achieved at 0.4 mol/L NaOH. Based on the above results, NaOH concentrations of 0.3, 0.4, and 0.5 mol/L were selected as the three levels for response surface optimization experiments.

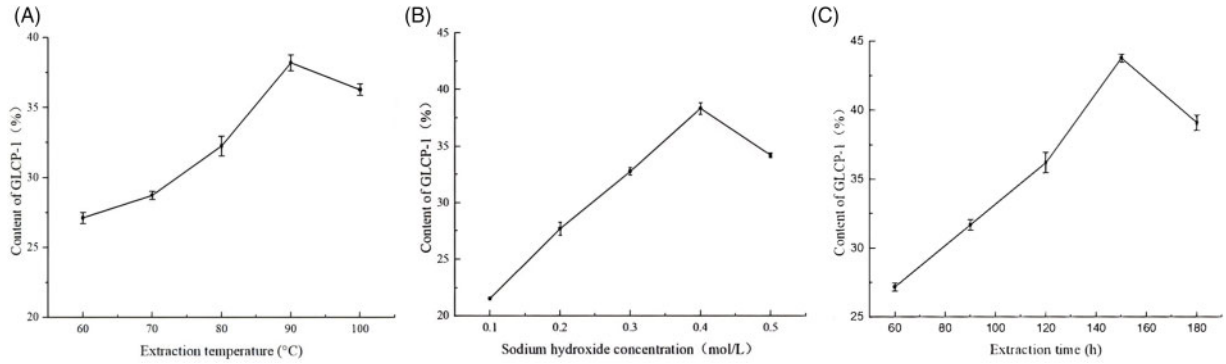

Figure 1. Single-factor analysis of alkali-extracted polysaccharides from *Ganoderma lucidum*: (**A**) Effect of NaOH concentration on polysaccharide content; (**B**) Effect of temperature on polysaccharide content; (**C**) Effect of extraction time on polysaccharide content

From Figure 1B, it can be concluded that the polysaccharide content extracted by the alkaline method first increases with rising temperature and reaches a peak at 90°C, then decreases. The reason may be that as the temperature increases, molecular thermal motion accelerates, which promotes the extraction of *Ganoderma lucidum* polysaccharides. However, when the temperature is too high, it leads to polysaccharide hydrolysis, causing the polysaccharide content to start decreasing [31]. 90°C is the temperature at which the polysaccharide content in the *Ganoderma lucidum* sample is the highest. Based on the results of the above experiments, temperatures of 80°C, 90°C, and 100°C are selected as the three levels for response surface optimization experiments.

From Figure 1C, it can be concluded that the polysaccharide content extracted by the alkaline method first increases with increasing extraction time and remains almost unchanged after 150 min. The extraction time of active ingredients is generally proportional to the extraction rate, meaning that as the extraction time increases, the extraction rate also generally increases [32–34]. Within a certain time range, increasing the extraction time promotes the extraction of *Ganoderma lucidum* polysaccharides. However, when the extraction time continues to increase, its effect on the polysaccharide content starts to decrease, possibly due to structural changes caused by prolonged heating of the polysaccharides [35]. Based on the results of the above experiments, extraction times of 120, 150, and 180 min are selected as the three levels for response surface optimization experiments.

3.2. Response surface experiment results

Based on the results of the single-factor experiments, the concentration of NaOH (*A*), temperature (*B*), and time (*C*) were fixed as the factors for investigation. The Box-Behnken design in the Design-Expert 8.0.6 software was used for experimental design. The levels and coding of the experimental factors are shown in Table 1, and the analysis of variance results for the regression model are shown in Table 2.

Table 2. Response surface experimental design scheme and results

S/N	*A* NaOH concentration mol/L	*B* Temperature/°C	*C* Time/min	*D* Polysaccharide content/%
1	0.4	90	150	45.3
2	0.4	80	180	39.4
3	0.5	100	150	38.2
4	0.3	80	150	34.1
5	0.5	90	180	41.2
6	0.5	90	120	38.2
7	0.5	80	150	33.7
8	0.3	90	120	39.1
9	0.4	90	150	45.3
10	0.4	90	150	45.3
11	0.4	90	150	45.3
12	0.3	100	150	36.2
13	0.4	100	120	39.7
14	0.4	90	150	45.3
15	0.4	80	120	37.6
16	0.4	100	180	43.3
17	0.3	90	180	39.7

3.2.1 Model establishment for investigated factors and Ganoderma lucidum polysaccharide content

Using DesignExpert 8.06 software to perform regression fitting analysis on thedata in Table 2, the following polynomial regression equation is obtained: $Y = 45.16 - 0.34A + 1.34B + 1.25C + 0.025AB + 0.100AC + 0.20BC - 3.69A^2 - 3.99B^2 - 1.42C^2$. Y: polysaccharide yield (%). The regression coefficients in the model were subjected to significance tests and variance analysis.

From Table 3, it can be seen that the experimental model shows an extremely significant difference ($p < 0.01$); the lack of fit term is not significant ($p = 0.1661 > 0.05$); the R^2 value is 0.9767, and the adjusted R^2 value is 0.9430. The quadratic terms A^2, B^2, and C^2 in the model are highly significant, indicating that the model fits the actual experimental results well, and the experimental error is small. The above regression equation can replace real experimental data. It is known that the order of the influence of each factor on *Ganoderma lucidum* polysaccharide content is: $B > A > C$, that is, Temperature > NaOH concentration > Time.

Table 3. Analysis of variance and significance test of response surface experiment results

Source of variance	Sum of squares	Degrees of freedom	Mean square	F value	*p* value
Model	173.76	9	19.31	381.22	<0.0001
A NaOH concentration	0.91	1	0.91	17.99	0.0038
B Temperature	14.31	1	14.31	282.59	<0.0001
C Time	12.50	1	12.50	246.83	<0.0001
AB	2.500E−003	1	2.500E−003	0.049	0.8305
AC	0.040	1	0.040	0.79	0.4037
BC	0.16	1	0.16	3.16	0.1187
A^2	57.41	1	57.41	1133.60	<0.0001
B^2	65.78	1	67.12	1325.28	<0.0001
C^2	7.99	1	8.46	167.06	<0.0001
Residual	0.35	7	0.051	–	–
Lack of fit	0.24	3	0.081	2.89	0.1661
Error term	0.51	4	0.13	–	–
Total	174.11	16	–	–	–

Note. $p < 0.05$, significant difference; $p < 0.01$, extremely significant difference.

3.2.2 Response surface analysis results

The shape of the response surface curves can reveal the significance level of the influencing factors [36]. Response surface plots were analyzed based on the interaction effects of temperature, NaOH concentration, and time. Based on the response surface plots and contour plots, the interactions between the variables can be further analyzed. The results are shown in Figure 2.

As shown in Figure 2A, the polysaccharide extraction yield gradually increases with elevated extraction temperature and NaOH concentration, reaching a peak before declining. The denser contour lines along the temperature axis compared to those along the NaOH concentration axis indicate that temperature exerts a more significant influence on the alkaline extraction yield of *Ganoderma lucidum* polysaccharides than NaOH concentration. The circular shape of the contour lines suggests insignificant interaction effects between extraction temperature and NaOH concentration.

Figure 2B demonstrates that the polysaccharide extraction yield remains relatively stable with prolonged extraction time but shows a rapid initial increase followed by a sharp decrease with increasing NaOH concentration. The contour plot of extraction time vs. NaOH concentration reveals sparser contour lines along the time axis compared to the NaOH concentration axis, indicating NaOH

concentration has a more pronounced effect on extraction efficiency than time. The elliptical contour lines suggest strong interactive effects between NaOH concentration and extraction time.

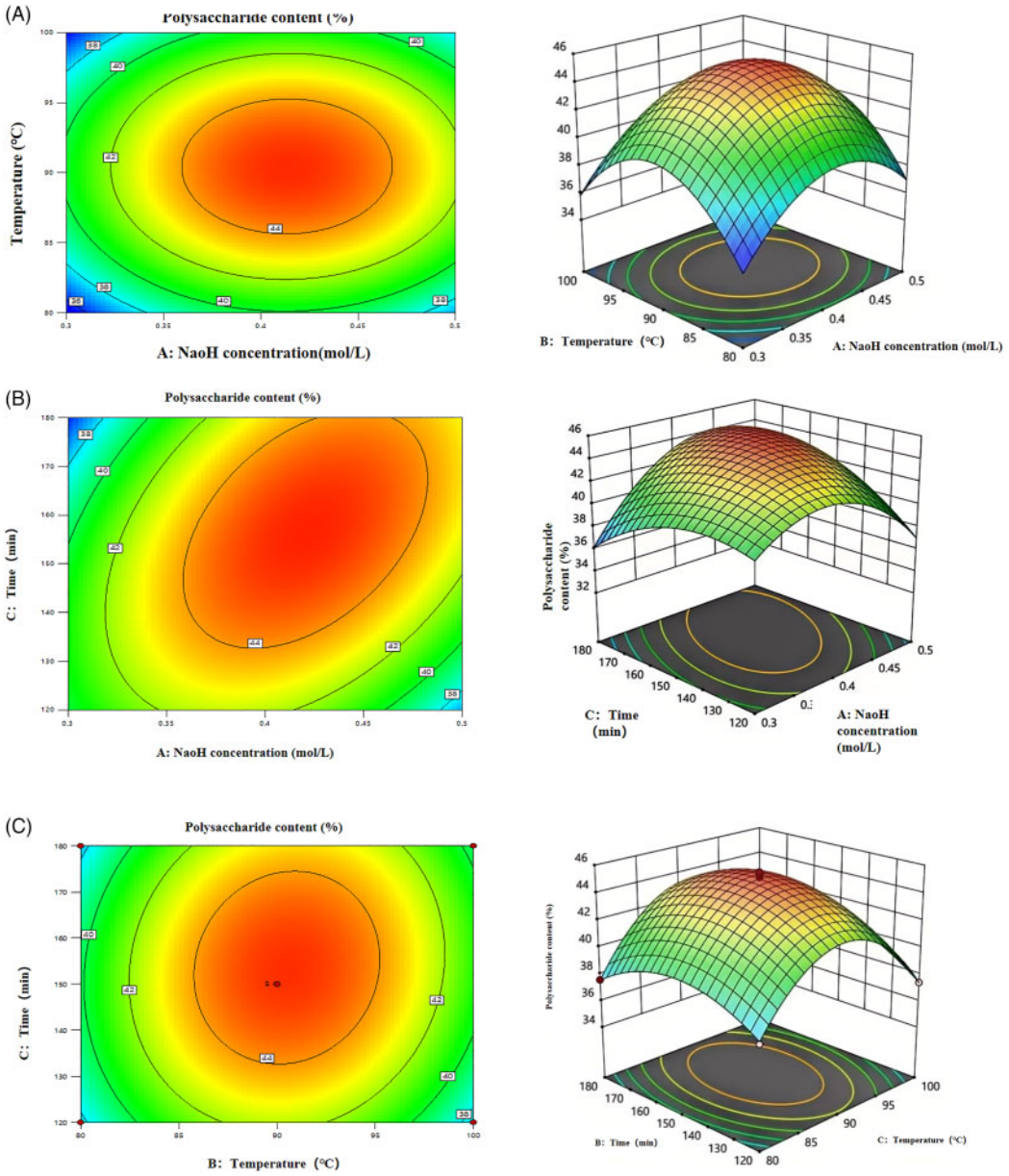

Figure 2. Optimization of the alkali extraction process for *Ganoderma lucidum* polysaccharides: (**A**) Effect of temperature and NaOH concentration on polysaccharide content; (**B**) Effect of time and NaOH concentration on polysaccharide content; (**C**) Effect of time and temperature on polysaccharide content

From Figure 2C, the polysaccharide extraction yield initially rises and then declines with increasing temperature, while showing minimal variation with extraction time. The sparser contour lines along the time axis relative to the temperature axis confirm the greater impact of temperature on extraction efficiency. The elliptical contour patterns further verify significant interaction effects between temperature and extraction time.

In summary, through analysis of the responses of three factors (NaOH concentration, extraction temperature, and time) and comparison of the three sets of response surface plots:

1. The steeper slopes observed in the temperature-NaOH concentration response surfaces
2. The gentler slopes in both temperature-time and time-NaOH concentration response surfaces

These patterns demonstrate that variations in *Ganoderma lucidum* polysaccharide content are more strongly influenced by temperature than time. Therefore, both temperature and NaOH concentration significantly affect the alkaline extraction efficiency of *Ganoderma lucidum* polysaccharides.

3.2.3 Verification Experiment

Based on a comprehensive analysis of the response surface experimental results and the regression model, the optimal process parameters for the alkaline extraction of *Ganoderma lucidum* polysaccharides were determined as follows: extraction temperature of 92.9°C, NaOH concentration of 0.41 mol/L, and extraction time of 172.36 min. Theoretically, the polysaccharide extraction yield under this optimized condition was calculated to be 40.8824%. To verify the reliability of the experiment and account for practical operability, the optimized parameters were adjusted to an extraction temperature of 93°C, NaOH concentration of 0.40 mol/L, and extraction time of 172 min. Three parallel experiments were conducted, yielding a polysaccharide extraction yield of 47.66%. These results provide valuable reference for the optimization of alkaline extraction processes for *Ganoderma lucidum* polysaccharides.

3.3. Total sugar content measurement

The total sugar content in the alkaline extract of *Ganoderma lucidum* was determined using the sulfuric acid-phenol method [37], and the standard curve is shown in Figure 3.

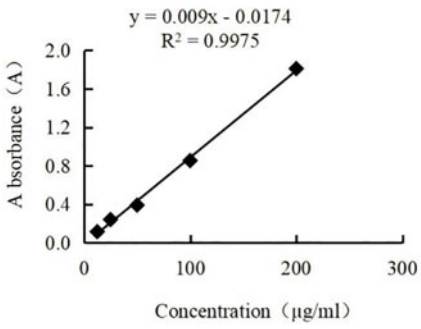

Figure 3. Standard curve of total sugar

The regression equation is $Y = 0.009X - 0.0174$, with $R^2 = 0.9975$, indicating a good linear relationship within the concentration range of 0~0.1 mg/mL. The average total sugar content calculated from the standard curve is 46.68%. Further studies on the fine structure and immunoactivity of the alkaline-extracted polysaccharides from *Ganoderma lucidum* will be conducted.

3.4. Results of uronic acid content determination

From Figure 4, the standard curve for uronic acid, the regression equation is $Y = 0.0048X + 0.0063$, with $r^2 = 0.9949$, indicating a good linear relationship within the concentration range of 0~0.1 mg/mL. Substituting the absorbance value of GLCP-1 into the regression equation, the uronic acid content was calculated to be 3.059%.

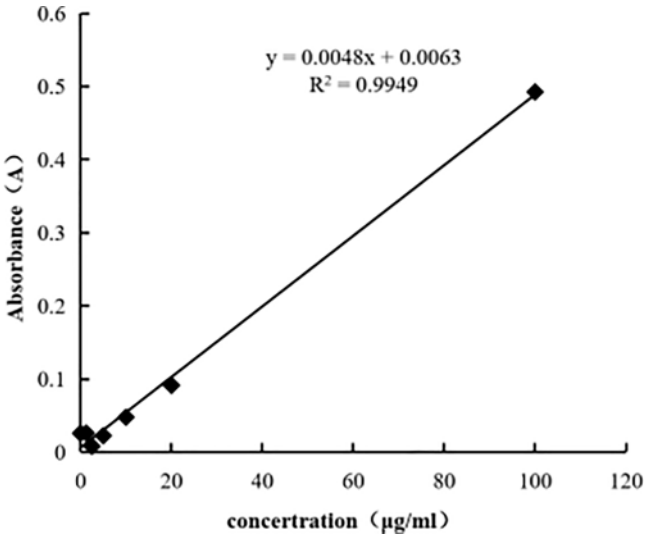

Figure 4. Standard curve of uronic acid

3.5. Analysis of monosaccharide results

The monosaccharide composition results are shown in Figure 5. After derivatization, the alkaline-extracted polysaccharides of *Ganoderma lucidum* were analyzed by HPLC. By comparing the peak positions of the mixed standard chromatogram with those of the extract under optimal conditions, the monosaccharide composition of the alkaline extract of *Ganoderma lucidum* was determined to be mannose, glucose, glucuronic acid, galactose, arabinose, and fucose. All target compounds in the sample were baseline-separated, with good resolution. There are some differences in monosaccharide composition depending on the extraction and measurement methods. The main chain of *Ganoderma lucidum* polysaccharides consists primarily of glucose, galactose, and mannose monosaccharides [27], which is consistent with the results of this experiment.Among these, mannose exhibits significant immunomodulatory effects. Studies demonstrate that mannose can activate macrophages and dendritic cells, thereby enhancing the body's immune response capabilities [38,39]. Glucose serves as the primary structural unit of polysaccharide backbones, and its content directly affects the structural stability and bioactivity of polysaccharides [40]. Glucuronic acid, a key component of acidic polysaccharides, displays notable antioxidant properties. Its carboxyl groups can scavenge free radicals, mitigating oxidative stress-induced cellular damage. Galactose demonstrates antitumor and anti-inflammatory effects by inhibiting tumor cell proliferation and inducing apoptosis. Arabinose, while a minor monosaccharide component in *Ganoderma lucidum* polysaccharides, contributes significantly to structural diversity and bioactivity. Fucose, though present in trace amounts within *Ganoderma lucidum* polysaccharides, exhibits remarkable antitumor activity and immunomodulatory effects [41,42].

3.6. Results of molecular weight determination

The results of molecular weight determination are shown in Figure 6. The relative molecular weight was detected using high-performance gel permeation chromatography (HPGPC), and the linear regression equation was $Y = -1.3909 + 19.455$. In Figure 6, the vertical axis Mw represents the average molecular weight (kDa), and the horizontal axis represents the retention time (min), with $R^2 = 0.996$. Based on the linear regression equation, the results are as follows: the average molecular weight of the alkaline-extracted polysaccharides from *Ganoderma lucidum* is 3.15×10^4 Da and 1.014×10^4 Da, indicating that the molecular weight of the alkaline-extracted polysaccharides is relatively small. Compared with the molecular weight of water-extracted Ganoderma polysaccharides [43,44], the molecular weight of the alkaline-extracted polysaccharides is higher, which may be related to the greater

degree of cell wall destruction by the alkaline extraction method. Further validation is needed to confirm the specific reasons.

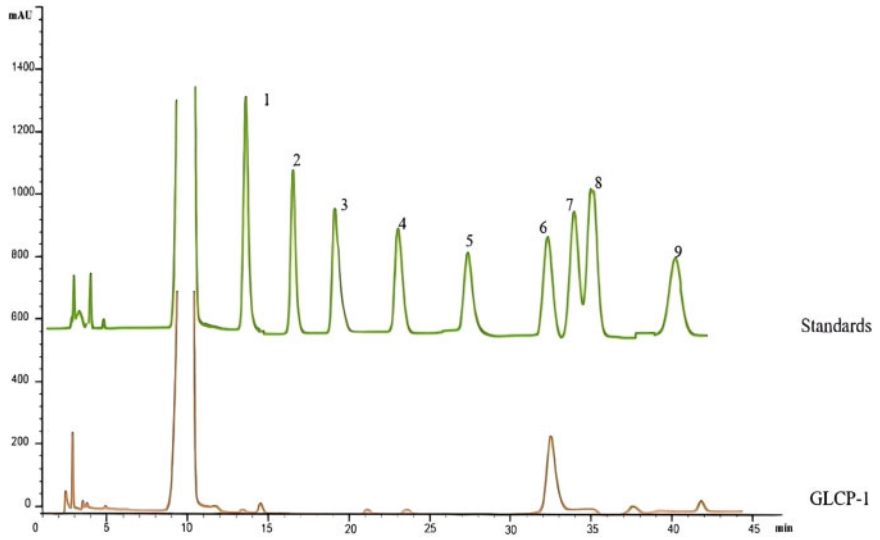

Figure 5. PMP-HPLC method for determining the chromatogram of monosaccharide composition (Note: 1. Mannose; 2. Rhamnose; 3. Glucuronic acid; 4. Galacturonic acid; 5. Glucose; 6. Galactose; 7. Xylose; 8. Arabinose; 9. Fucose)

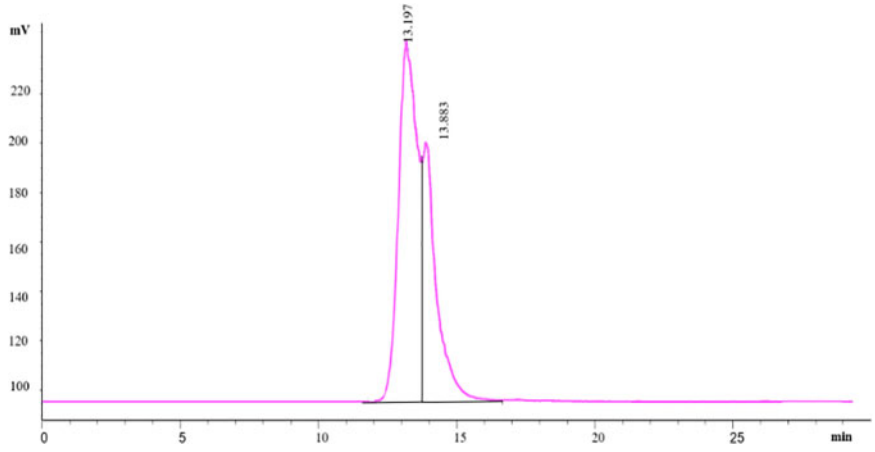

Figure 6. Chromatogram of HCGPC method for determining the molecular weight of polysaccharides

3.7. Results of infrared spectroscopy scanning

The infrared spectroscopy analysis is shown in Figure 7. The results show a broad and strong characteristic absorption peak of polysaccharides at 3392.77 cm^{-1}, caused by O-H stretching vibrations. A characteristic absorption peak of polysaccharides appears at 1387.76 cm^{-1}, caused by C-H deformation vibrations [44]. Between 1300 and 950 cm^{-1}, there are mainly absorption peaks for various C-O stretching vibrations, which are polysaccharide absorption peaks [45]. Additionally, signal peaks appear between 900 and 800 cm^{-1}, indicating that the alkaline-extracted *Ganoderma lucidum* polysaccharides are connected by β-glycosidic bonds [46]. The strong absorption peak of the O-H stretching vibration in the infrared spectrum indicates the abundant presence of hydroxyl (-OH) groups in *Ganoderma lucidum*

polysaccharides [47]. Hydroxyl groups are not only key contributors to the polarity and water solubility of polysaccharide molecules but also participate in hydrogen bond networks that enhance antioxidant capacity by facilitating free radical scavenging. The presence of β-glycosidic bonds may influence the spatial conformation of the polysaccharides, thereby modulating their interaction with biological receptors. β-glycosidic bonds are often associated with specific bioactivities, such as antitumor activity or immunomodulatory effects [48].

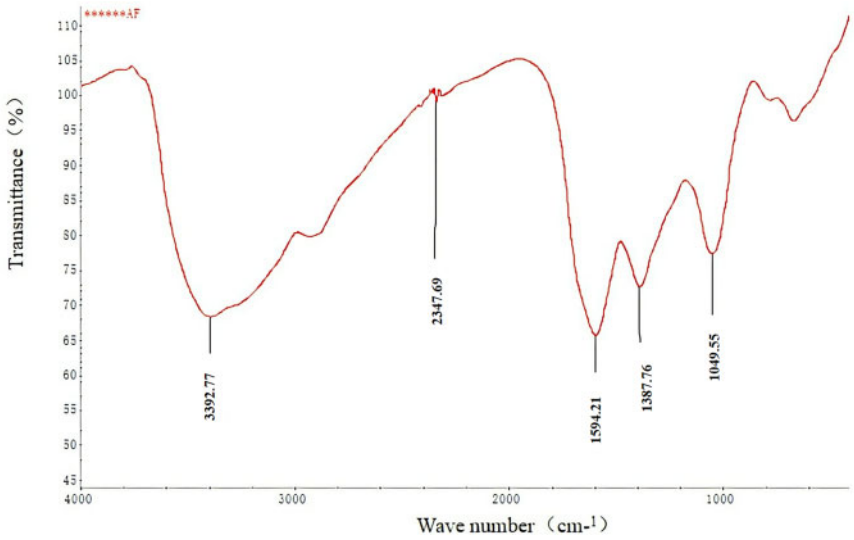

Figure 7. Infrared spectrum of alkaline extracted polysaccharides from *Ganoderma lucidum*

3.8. Results of RAW264.7 cell viability assay

The results in the Figure 8 show that after treating RAW 264.7 cells with *Ganoderma lucidum* polysaccharides for 3 days, cell viability increases with the increase in *Ganoderma lucidum* polysaccharide concentration. The experiment shows that, compared with the control group, GLCP-1 has a good protective effect on RAW 264.7 cells.

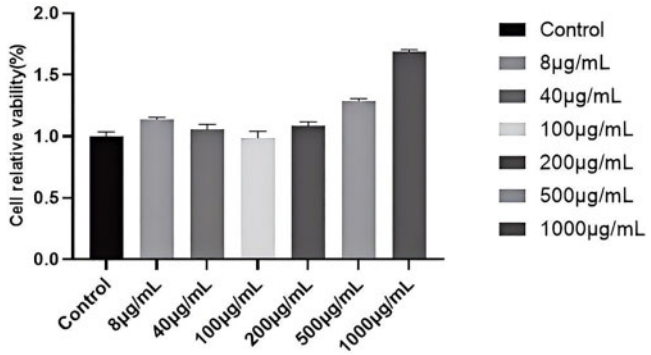

Figure 8. Effect of different concentrations of *Ganoderma lucidum* polysaccharides on RAW264.7 cell viability

3.9. Results of CCK-8 viability assay

From Figure 9, the CCK-8 results show that *Ganoderma lucidum* polysaccharides at concentrations of 8, 100, 200, 500, and 1000 μg/mL significantly inhibit the viability of HepG2 cells compared with

the control group. When the concentration of *Ganoderma lucidum* polysaccharides reaches 100, 200, and 500 μg/mL, cell viability significantly decreases. Except for the 40 μg/mL concentration, other differences are statistically significant.

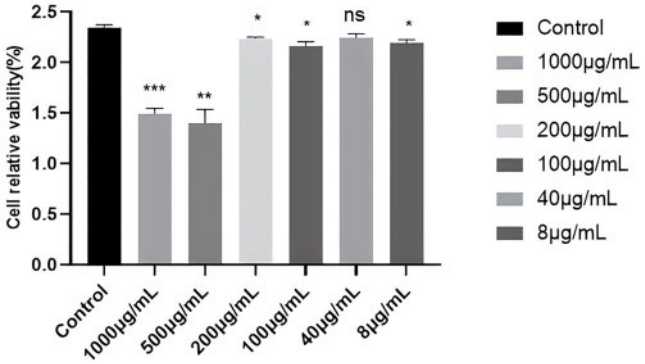

Figure 9. Effect of different concentrations of *Ganoderma lucidum* polysaccharides on HepG2 cell viability. Note: ns: Not statistically significant ($p \geq 0.05$), $^{*}p < 0.05$: Statistically significant at the 0.05 level $^{**}p < 0.01$, $^{***}p < 0.001$

4. Conclusion

In this experiment, the alkaline extraction method was used to perform reflux extraction on *Ganoderma lucidum*, and single-factor experiments and response surface experiments were designed. Based on the data from the single-factor experiments, response surface experiments were designed to determine the polysaccharide content of *Ganoderma lucidum*. The data were input into DesignExpert 8.06 software, and the optimal process for alkaline extraction of *Ganoderma lucidum* polysaccharides was determined to be: temperature 92.9°C, extraction time 172.36 min, and NaOH concentration 0.41 mol/L. The polysaccharide content of *Ganoderma lucidum* was 45.37%. The process conditions were adjusted to: temperature 93°C, extraction time 172 min, and NaOH concentration 0.40 mol/L. Under these conditions, the total sugar content of *Ganoderma lucidum* polysaccharides was determined to be 47.66%. Using the PMP-HPLC method, the monosaccharide composition of the *Ganoderma lucidum* extract was determined to include mannose, glucuronic acid, glucose, galactose, arabinose, and fucose. The molecular weight of the polysaccharides was determined using HCGPC to be 3.15×10^4 Da and 1.014×10^4 Da. Infrared spectroscopy showed that the alkaline-extracted *Ganoderma lucidum* polysaccharides are connected by β-glycosidic bonds. The RAW264.7 cell viability assay showed that the alkaline-extracted *Ganoderma lucidum* polysaccharides could enhance cell viability and reduce cell damage. The CCK-8 cell viability assay showed that the alkaline-extracted *Ganoderma lucidum* polysaccharides have the effect of inhibiting the proliferation of liver cancer cells. The optimized extraction conditions exhibit high stability and practicability, making them suitable for large-scale industrial production. Precise control of temperature, time, and NaOH concentration helps ensure product quality consistency while reducing batch-to-batch variations. Although this study has achieved significant findings, numerous aspects require further investigation to fully realize the potential of *Ganoderma lucidum* polysaccharides and provide comprehensive scientific support for practical applications. Future research could employ advanced techniques such as nuclear magnetic resonance (NMR) and mass spectrometry (MS) to elucidate the precise structural characteristics of these polysaccharides, thereby establishing a more accurate molecular basis for functional studies. While the current work preliminarily confirmed the immunomodulatory effects of alkali-extracted Ganoderma polysaccharides, the underlying molecular mechanisms remain unclear. Subsequent investigations could employ cell signaling pathway analysis and animal model experiments to systematically explore these mechanisms,

providing stronger theoretical support for pharmaceutical applications. Additionally, the study utilized crude polysaccharide extracts without further purification or modification. Future work could implement chromatographic separation techniques for purification and explore chemical modifications (e.g., sulfation, acetylation) to alter physicochemical properties and investigate functional activity variations, thereby expanding potential pharmaceutical applications. This study not only optimized the extraction process of *Ganoderma lucidum* polysaccharides but also revealed their structural characteristics through multiple analytical approaches (e.g., monosaccharide composition, molecular weight, and infrared spectroscopy), providing a scientific basis for their development and application. Furthermore, cell experiments have shown that alkali-extracted *Ganoderma lucidum* polysaccharides have an obvious protective effect on cells and a significant inhibitory effect on cancer cells, laying the groundwork for their potential applications in functional foods, nutraceuticals, and pharmaceutical development.

5. Summary

The study optimized the alkaline extraction process of *Ganoderma lucidum* polysaccharides, identifying optimal conditions for temperature, NaOH concentration, and extraction time, and analyzing their monosaccharide composition, molecular weight, and immunological activity. The polysaccharides demonstrated potential biological activity, enhancing RAW264.7 cell viability and inhibiting HepG2 cells, providing a basis for future applications and industrial production.

Acknowledgement: We would like to thank the Department of Pharmacognosy at the College of Pharmacy, Jiamusi University, for providing resources and facilities essential to this research. Additionally, we extend our gratitude to the staff at Jiamusi Traditional Chinese Medicine Hospital for their support in sourcing *Ganoderma lucidum*.

Funding Statement: The authors received no specific funding for this study.

Author Contributions: The authors confirm contribution to the paper as follows: Conceptualization, Chuanli Jin and Ruian Ma; investigation, Chen Chen and Ze Dang; data curation, Chen Chen and Ze Dang; formal analysis, Tong Yu and Yu Fu; writing—original draft preparation, All authors; writing—review and editing, Juan Du; supervision, Juan Du; project administration, Juan Du. All authors reviewed the results and approved the final version of the manuscript.

Availability of Data and Materials: The data that support the findings of this study are available from the corresponding author, Juan Du, upon reasonable request.

Ethics Approval: Not applicable.

Conflicts of Interest: The authors declare no conflicts of interest to report regarding the present study.

Abbreviation

1. NaOH Sodium Hydroxide
2. GLCP-1 *Ganoderma lucidum* Polysaccharide 1
3. RAW264.7 A macrophage cell line (Mouse leukemic monocyte/macrophage cell line)
4. CCK8 Cell Counting Kit-8
5. HepG2 Human liver cancer cell line

References

1. Chinese Pharmacopoeia Commission. Pharmacopoeia of the People's Republic of China. Vol. 1. Beijing, China: China Medical Science Press; 2020.
2. Zeng M, Xie MJ, Zhu ZY, Yu YT, Zhang M. Quality evaluation of *Ganoderma lucidum* from different areas. Chin Tradit Herb Drugs. 2023;54(21):7193–201. (In Chinese). doi:10.7501/j.issn.0253-2670.2023.21.028.

3. Wu M, Zhang S, Jing L, Lv F. Research progress on active components and pharmacological effects of *Ganoderma lucidum*. Forest Byprod Spec China. 2023;2:76–9. (In Chinese).
4. Gao S, Wang Y, Shan Y, Wang W, Li J, Tan H. *Rhizoma Coptidis* polysaccharides: extraction, separation, purification, structural characteristics and bioactivities. Int J Biol Macromol. 2025;320(Pt 1):145677. doi:10.1016/j.ijbiomac.2025.145677.
5. Zhang RB, Yang YH, Li CX, Li WL. Research progress on pharmacological action and mechanism of *Ganoderma lucidum* polysaccharides. Nat Prod Res Dev. 2023;35(5):879–87. (In Chinese). doi:10.16333/j.1001-6880.2023.5.016.
6. Li J, Cai C, Zheng M, Hao J, Wang Y, Hu M, et al. Alkaline extraction, structural characterization, and bioactivities of (1→6)-β-d-glucan from *Lentinus edodes*. Molecules. 2019;24(8):1610. doi:10.3390/molecules24081610.
7. Shu YT, Gao J, Peng WX, Li Y, Gu W, Dong J, et al. Study on antioxidation of *Ganoderma lucidum* polysaccharide. J Nanjing Univ Tradit Chin Med. 2020;36(4):504–8. (In Chinese). doi:10.14148/j.issn.1672-0482.2020.0504.
8. Li M, Yu L, Zhai Q, Liu B, Zhao J, Zhang H, et al. *Ganoderma applanatum* polysaccharides and ethanol extracts promote the recovery of colitis through intestinal barrier protection and gut microbiota modulations. Food Funct. 2022;13(2):688–701. doi:10.1039/d1fo03677g.
9. Do DT, Lam DH, Nguyen T, Phuong Mai TT, Phan LTM, Vuong HT, et al. Utilization of response surface methodology in optimization of polysaccharides extraction from Vietnamese red *Ganoderma lucidum* by ultrasound-assisted enzymatic method and examination of bioactivities of the extract. Sci World J. 2021;2021(8):7594092. doi:10.1155/2021/7594092.
10. Tian B, Zhou X, Geng Y, Hu J, Ye B, Sun P, et al. Characterization and *in vitro* digestion of alkali-extracted polysaccharides from *Grifola frondosa* and its impacts on human gut microbiota. Food Biosci. 2024;60(2):104499. doi:10.1016/j.fbio.2024.104499.
11. Wang W, Tan J, Nima L, Sang Y, Cai X, Xue H. Polysaccharides from fungi: a review on their extraction, purification, structural features, and biological activities. Food Chem X. 2022;15(12):100414. doi:10.1016/j.fochx.2022.100414.
12. Zhang J, Ye Z, Liu G, Liang L, Wen C, Liu X, et al. Subcritical water enhanced with deep eutectic solvent for extracting polysaccharides from *Lentinus edodes* and their antioxidant activities. Molecules. 2022;27(11):3612. doi:10.3390/molecules27113612.
13. Li H, Tao W, Xu X, Chen G, Ma W, Jia S. Lycium barbarum polysaccharides alleviate pancreatic β-cells apoptosis through the inhibition of IFNγ pathway. J Funct Foods. 2023;107(9):105706. doi:10.1016/j.jff.2023.105706.
14. Wei Q, Zhang YH. Ultrasound-assisted polysaccharide extraction from *Cercis chinensis* and properites, antioxidant activity of polysaccharide. Ultrason Sonochem. 2023;96(1):106422. doi:10.1016/j.ultsonch.2023.106422.
15. Yan X, Miao J, Zhang B, Liu H, Ma H, Sun Y, et al. Study on semi-bionic extraction of Astragalus polysaccharide and its anti-aging activity *in vivo*. Front Nutr. 2023;10:1201919. doi:10.3389/fnut.2023.1201919.
16. Li ZY, Zhang YX, Zhao XX, Wang YL, Li YL, Gong AQ. Research progress on extraction and biological activity of Chinese yam polysaccharides. Inn Mong Agric Sci Technol. 2024;52(5):129–34. (In Chinese). doi:10.12190/j.issn.2096-1197.2024.05.13.
17. Gu FF, Li J, Yang CD, Hu MH, Fan LD, Hao JJ, et al. Fractionation, structural characterization, and immunomodulatory activity of polysaccharide isolated from *Ganoderma lucidum*. Chin Tradit Herb Drugs. 2018;49(10):2359–64. (In Chinese). doi:10.7501/j.issn.0253-2670.2018.08.017.
18. Huang SQ, Li JW, Wang Z, Pan HX, Chen JX, Ning ZX. Optimization of alkaline extraction of polysaccharides from *Ganoderma lucidum* and their effect on immune function in mice. Molecules. 2010;15(5):3694–708. doi:10.3390/molecules15053694.

19. Mat Yusoff M, Gordon MH, Niranjan K. Aqueous enzyme assisted oil extraction from oilseeds and emulsion de-emulsifying methods: a review. Trends Food Sci Technol. 2015;41(1):60–82. doi:10.1016/j.tifs.2014.09.003.
20. Zhang Z, Yi Y, Peng K, Liu Y, Yan Z. Optimization of alkaline extraction process of *Nelumbo nucifera* polysaccharides and evaluation of antioxidant activity. Food Saf Qual. 2023;14(2):256–63. (In Chinese).
21. Gu H, Liang L, Zhu XP, Jiang X, Du M, Wang Z. Optimization of enzymatic extraction, characterization and bioactivities of Se-polysaccharides from Se-enriched *Lentinus edodes*. Food Biosci. 2023;51(17):102346. doi:10.1016/j.fbio.2022.102346.
22. Chen W, Ma X, Jin W, Cheng H, Xu G, Wen H, et al. Shellfish polysaccharides: a comprehensive review of extraction, purification, structural characterization, and beneficial health effects. Int J Biol Macromol. 2024;279(Pt 3):135190. doi:10.1016/j.ijbiomac.2024.135190.
23. Li H, Li C, Xu Y, Cao H, Wang X, He J. Ultrasonic-assisted alkali extraction of quinoa polysaccharides: yield and structural characterization. J Cereal Sci. 2025;122:104108. doi:10.1016/j.jcs.2025.104108.
24. Dai J, Wu Y, Chen SW, Zhu S, Yin HP, Wang M, et al. Sugar compositional determination of polysaccharides from *Dunaliella salina* by modified RP-HPLC method of precolumn derivatization with 1-phenyl-3-methyl-5-pyrazolone. Carbohydr Polym. 2010;82(3):629–35. doi:10.1016/j.carbpol.2010.05.029.
25. Yang Y, Li W, Guo L, Liu Y, Tang Q, Zhang J. Determination of polysaccharide molecular weight, polysaccharide composition, and monosaccharide content in *Antrodia cinnamomea* fruiting bodies using HPGPC combined with HPLC-ELSD. J Chin Med Mater. 2018;41(5):1145–7. (In Chinese). doi:10.13863/j.issn1001-4454.2018.05.028.
26. Li J, Zhang ZQ, Chen H. Analysis of *Flammalina velutipes* polysaccharide by gas chromatography and infrared spectra. Sci Technol Food Ind. 2010;31(9):147–9. (In Chinese). doi:10.13386/j.issn1002-0306.2010.09.101.
27. Huang F, Zhang R, Yi Y, Tang X, Zhang M, Su D, et al. Comparison of physicochemical properties and immunomodulatory activity of polysaccharides from fresh and dried litchi pulp. Molecules. 2014;19(4):3909–25. doi:10.3390/molecules19043909.
28. Liu S, Cao X, Xu L, Bai Y, Lu L, Chai Y. Study on the optimal conditions for detecting the activity of chicken lymphocytes using the CCK8 method. Heilongjiang Anim Sci Vet Med. 2017;2017(13):212–4. (In Chinese). doi:10.13881/j.cnki.hljxmsy.2017.1185.
29. Huang H, Huang G. Extraction, separation, modification, structural characterization, and antioxidant activity of plant polysaccharides. Chem Biol Drug Des. 2020;96(5):1209–22. doi:10.1111/cbdd.13794.
30. Shida M, Ushioda Y, Nakajima T, Matsuda K. Structure of the alkali-insoluble skeletal glucan of Lentinus edodes. J Biochem. 1981;90(4):1093–100. doi:10.1093/oxfordjournals.jbchem.a133561.
31. Chen L, Zheng TT, Zhu HY, Wang WD, Sun YE, Gong H. Optimization of ultrasound-assisted extraction of polysaccharides from *Agaricus blazei* by response surface methodology and its *in vitro* antioxidant activity. China Food Addit. 2024;35(1):143–9. (In Chinese). doi:10.19804/j.issn1006-2513.2024.1.017.
32. Bai L, Zhu P, Wang W, Wang M. The influence of extraction pH on the chemical compositions, macromolecular characteristics, and rheological properties of polysaccharide: the case of okra polysaccharide. Food Hydrocoll. 2020;102(1):105586. doi:10.1016/j.foodhyd.2019.105586.
33. Zhang Z, Zheng YJ, Ji SQ, Ni QX, Zhang YZ, Xu GZ. Optimization of extraction process of Gardenia flower pectin polysaccharide by response surface method. China Food Addit. 2021;32(11):45–52. (In Chinese). doi:10.19804/j.issn1006-2513.2021.11.007.

34. Mittal A, Katahira R, Donohoe BS, Pattathil S, Kandemkavil S, Reed ML, et al. Ammonia pretreatment of corn stover enables facile lignin extraction. ACS Sustainable Chem Eng. 2017;5(3):2544–61. doi:10.1021/acssuschemeng.6b02892.
35. Shi JX, Xu XM, Yu JN. Review: extraction method, structural identification and pharmacological activity of *Polygonatum sibiricum* polysaccharide. Chin Wild Plant Resour. 2019;38(2):36–42. (In Chinese). doi:10.3969/j.issn.1006-9690.2019.02.008.
36. Yang Q, Cai XX, Guo LK, Liu ZY, Wang SY. Optimization of protein extraction from *Chlorella vulgaris* using respone surface methodology and study on its property. J Chin Inst Food Sci Technol. 2018;18(6):183–90. (In Chinese). doi:10.16429/j.1009-7848.2018.06.024.
37. Wang Y, Lou F, Chen Y, Yang H, Zhao Z, Jia Y, et al. Optimization of conditions for determining polysaccharide content in *Dioscorea alata* using the phenol-sulfuric acid method. Food Res Dev. 2021;42(4):170–4. doi:10.12161/j.issn.1005-6521.2021.04.029.
38. Wang TT, Chen TX, Xiao JY, Yu GJ, Lin YF, Wu JY, et al. Progress of functional research on the polysaccharide activity of *Ganoderma lucidum*. Edible Fungi China. 2022;41(1):7–16. (In Chinese). doi:10.13629/j.cnki.53-1054.2022.01.002.
39. Wang W, Wang J, Dong SF, Liu CH, Italiani P, Sun SH, et al. Immunomodulatory activity of andrographolide on macrophage activation and specific antibody response. Acta Pharmacol Sin. 2010;31(2):191–201. doi:10.1038/aps.2009.205.
40. Hassan I, Gani A, Ahmad M, Banday J. Extraction of polysaccharide from *Althea rosea* and its physicochemical, anti-diabetic, anti-hypertensive and antioxidant properties. Sci Rep. 2022;12(1):17116. doi:10.1038/s41598-022-20134-6.
41. Chen H, Liu N, He F, Liu Q, Xu X. Specific β-glucans in chain conformations and their biological functions. Polym J. 2022;54(4):427–53. doi:10.1038/s41428-021-00587-8.
42. Wei J, Dai Y, Zhang N, Wang Z, Tian X, Yan T, et al. Natural plant-derived polysaccharides targeting macrophage polarization: a promising strategy for cancer immunotherapy. Front Immunol. 2024;15:1408377. doi:10.3389/fimmu.2024.1408377.
43. Xiong C, Luo Q, Jin X, Chen C, Li Q, Chen ZQ, et al. Physiochemical properties immunoregulatory effects of the polysaccharides from *Ganoderma lingzhi*. Microbiol China. 2018;45(4):825–35. (In Chinese). doi:10.13344/j.microbiol.china.170415.
44. Guo C, Li C, Hou MM, Bai MY, Zhou CC, Zhang HY. Extraction optimization and its inoxidizability and hypoglycemic properties *in vitro* of polysaccharide from *Salvia plebeia* R. Br Sci Technol Food Ind. 2022;43(20):211–9. (In Chinese). doi:10.13386/j.issn1002-0306.2021120280.
45. Thombare N, Mahto A, Singh D, Chowdhury AR, Ansari MF. Comparative FTIR characterization of various natural gums: a criterion for their identification. J Polym Environ. 2023;31(8):3372–80. doi:10.1007/s10924-023-02821-1.
46. Liu JJ, Chen SK, Wang X, He WW, Song XX, Huang XJ, et al. Changes of the physicochemical properties and structural characteristics of alkali-extracted polysaccharides from *Agrocybe cylindracea* across the growth process. J Agric Food Chem. 2024;72(22):12810–21. doi:10.1021/acs.jafc.4c02218.
47. He J, Shao P, Men X, Sun P. Analysis of structural characteristics of polysaccharide from *Ganoderma lucidum*. Chinese J Analyt Chem. 2010;38(3):372–6. doi:10.3724/SP.J.1096.2010.00372.
48. Wu ZW, Zhao XF, Quan CX, Liu XC, Tao XY, Li YJ, et al. Structure-function insights of natural *Ganoderma* polysaccharides: advances in biosynthesis and functional food applications. Nat Prod Bioprospect. 2025;15(1):15. doi:10.1007/s13659-025-00496-w.

Received: 29 May 2025; Accepted: 26 August 2025; Published: 30 September 2025