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Optimization of Polysaccharides Extraction from *Physalis alkekengi L.* Peel and Its Effect on the Expression of Inflammation-Related Proteins in SW620 Cells

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Abstract: *Objective:* To establish an optimized aqueous extraction process for polysaccharides from *Physalis alkekengi L.* peel and to preliminarily explore its *in vitro* anti-inflammatory activity against colorectal cancer SW620 cells. *Methods:* A single-factor test combined with orthogonal test analysis was used to evaluate the effects of the material-to-liquid ratio, extraction temperature, and extraction time on the yield of polysaccharides from *Physalis alkekengi L.* peel. The antioxidant activity of the polysaccharides was assessed by analyzing their free radical scavenging ability *in vitro*, and the anti-inflammatory effect was evaluated using SW620 cells. *Results:* The optimal extraction conditions were a material-to-liquid ratio of m(g):V(mL) = 1:30, an extraction temperature of 100°C, and an extraction time of 40 minutes, with a predicted polysaccharide yield of 25.7%. The polysaccharides from Physalis peruviana peel effectively scavenged DPPH, superoxide anion, and hydroxyl radicals. After treatment with Physalis peruviana polysaccharides, the levels of IL-1β, IL-18, and TNF-α in the cell culture medium were significantly reduced, and the phosphorylation level of P65 protein in SW620 cells was decreased. *Conclusion:* This extraction method is stable and reliable, and the prepared *Physalis alkekengi L.* polysaccharides exhibit significant *in vitro* antioxidant and anti-inflammatory activities. This study provides a theoretical basis for developing drugs for the prevention and treatment of colorectal cancer.

Keywords: *Physalis alkekengi L.* polysaccharide; Antioxidant; IL-1β; Extraction process; Colorectal cancer

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1. Introduction

Colorectal cancer (CRC) is the third most prevalent cancer worldwide. Despite a decline in the incidence of colon cancer over the past few decades, it remains one of the leading causes of cancer mortality and morbidity globally, posing a significant public health challenge [1]. Polysaccharides are polymers composed of aldose or ketose linked by glycosidic bonds and form a fundamental component of all living organisms, being

involved in various physiological functions [2]. Polysaccharides from traditional Chinese medicinal herbs exhibit pharmacological effects such as antioxidant, antitumor, antiviral, lipid-lowering, and blood glucose-lowering activities. In recent years, researchers have conducted antioxidant studies on hundreds of herbal monomers, active sites, and compound preparations, with polysaccharides showing strong antioxidant activity as biomacromolecules [3].

Physalis alkekengi L. belongs to the *Physalis* genus, and some secondary metabolites in this plant give it commercial value. Many compounds in *Physalis* have antibacterial, anti-inflammatory, and anticancer properties, making them valuable in the medical field. The whole plant of *Physalis* is used in traditional medicine to treat fever, diabetes, pharyngitis, boils, coughs, and mastitis. The main active components are saponins, polysaccharides, flavonoids, and others [4], among which polysaccharides from *Physalis alkekengi L.* possess anti-inflammatory, antitumor, and antioxidant effects [5]. It is hypothesized that the polysaccharides in *Physalis alkekengi L.* peel may have antioxidant activity, potentially offering therapeutic benefits for CRC treatment.

Inflammatory responses are involved in every stage of CRC development. This study uses single-factor and orthogonal test designs to optimize the extraction process and evaluate the free radical scavenging ability of *Physalis alkekengi L.* polysaccharides. The study also investigates the mechanism of action of these polysaccharides on inflammation in SW620 cells, providing a theoretical basis for developing targeted anti-inflammatory therapeutic strategies for CRC.

2. Materials and methods

2.1. Main materials

Physalis alkekengi L. peels were purchased by students from a Physalis production base in their hometown; Coomassie Brilliant Blue and ascorbic acid were purchased from Beijing Dingguo Changsheng Biotechnology Co., Ltd.; dihydroxybenzene was obtained from Shanghai Yuan Ye Biological Technology Co., Ltd.; colorectal cancer SW620 cells were procured from Wuhan Procell Life Technology Co., Ltd.; fetal bovine serum and DMEM medium were from Gibco, USA; P65, p-P65, and β-actin antibodies were from Cell Signaling Technology, USA; IL-1β, IL-18, and TNF-α ELISA kits were from Wuhan Boster Biological Technology Co., Ltd.

2.2. Experimental methods

2.2.1. Single-factor extraction of polysaccharides from Physalis alkekengi L. peel

The *Physalis alkekengi L.* peel was washed, dried, and crushed through an 80-mesh sieve to obtain a fine powder. The powder was subjected to aqueous extraction under different conditions (material-to-liquid ratio, extraction temperature, and extraction time). The extraction solution was centrifuged, rotary evaporated, and concentrated to a certain volume, then precipitated with four times the volume of 95% ethanol at 4°C overnight and centrifuged at 5000 r/min for 20 minutes. Each sample was prepared and measured. Different extraction factors were set, and each experiment was repeated three times.

2.2.2. Orthogonal experiment

Based on the results of the single-factor experiments, an orthogonal experiment was designed, selecting A (material-to-liquid ratio [m(g):V(mL)]), B (extraction temperature [°C]), and C (extraction time [h]) for three-factor, three-level response conditions.

2.2.3. Process verification

Based on the optimized conditions obtained from the orthogonal experiment, three parallel experiments were conducted. The extracted polysaccharides were concentrated and freeze-dried to obtain refined *Physalis alkekengi L.* polysaccharides. Polysaccharide yield was calculated as (mass of refined polysaccharides/ mass of raw material) × 100%. This determined the optimal extraction process for *Physalis alkekengi L.* polysaccharides.

2.2.4. In vitro antioxidant activity of Physalis alkekengi L. polysaccharides

A stock solution of *Physalis alkekengi L.* polysaccharides was prepared at a concentration of 1 mg/mL. Aliquots of 0.2, 0.5, 1, 2, 4, and 6 mL of the stock solution were taken into test tubes, and distilled water was used to adjust each group to a final volume of 1 mL. VC served as a positive control. The DPPH radical scavenging rate, superoxide anion radical scavenging rate, and hydroxyl radical elimination rate of the polysaccharides were measured using a UV spectrophotometer.

2.2.5. Effect of Physalis alkekengi L. polysaccharide on SW620 cell viability

SW620 and 293 cells were seeded at a density of 2×10^5/mL in 96-well plates, with 100 μL per well, and cultured overnight. Cells were divided into a normal control group, an *Physalis alkekengi L.* polysaccharide control group, and groups with varying concentrations of *Physalis alkekengi L.* polysaccharides plus SW620/293 cells. After 24 hours of culture, 10 μL/well of CCK8 reagent was added, and absorbance was measured at 450 nm. Cell viability was calculated as (mean value of experimental wells/mean value of control wells) × 100%.

2.2.6. Western blot experiment

Total protein was extracted from each group of cells, and protein concentration was measured. SDS-PAGE electrophoresis was performed using 50 μg/well, followed by membrane transfer, blocking, primary antibody incubation, secondary antibody incubation, and ECL chemiluminescence detection.

2.2.7. ELISA experiment

Cell culture supernatants from each group were collected to measure the expression levels of IL-1β, IL-18, and TNF-α.

2.3. Statistical analysis

Data were processed and analyzed using SPSS 25.0. Results were expressed as mean ± standard deviation (SD); variance analysis was performed for multiple groups, and Fisher's LSD test was conducted for pairwise comparisons between groups. Differences were considered statistically significant at $P < 0.05$.

3. Results and analyses

3.1. Single-factor experiment results for polysaccharide extraction from Physalis alkekengi L. peel

The results of this study showed that in experiments with different influencing factors, the extraction rate of polysaccharides from *Physalis alkekengi L.* peels initially increased and then decreased. When the material-to-liquid ratio m(g):V(mL) was increased to 1:30, the polysaccharide yield began to slowly decrease. The polysaccharide yield decreased as the extraction temperature increased beyond 90°C, so 90°C was selected as

the optimal extraction temperature. The polysaccharide yield reached its maximum at an extraction time of 30 minutes, after which it decreased, so 30 minutes was chosen as the optimal extraction time, as shown in **Figure 1**.

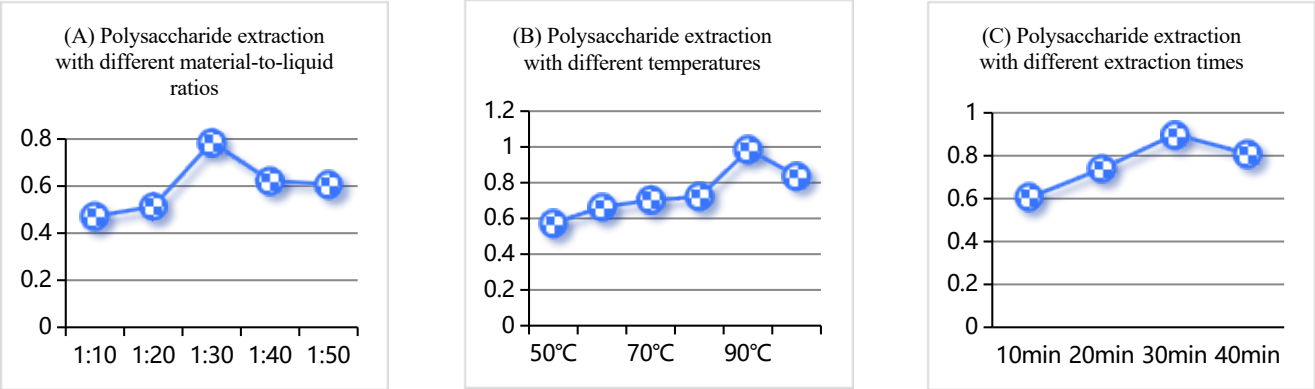

Figure 1. Single-factor extraction of polysaccharides from *Physalis alkekengi L.* peels with different **(A)** material-to-liquid ratios, **(B)** temperatures, and **(C)** extraction times.

3.2. Orthogonal experiment results for polysaccharide extraction from Physalis alkekengi L. peels

The orthogonal experiment, conducted under 3-factor and 3-level response conditions, determined the optimal extraction process for polysaccharides from *Physalis alkekengi L.* peels: a material-to-liquid ratio of m(g):V(mL) = 1:30, an extraction temperature of 100°C, and an extraction time of 40 minutes, with a predicted polysaccharide yield of 25.7%, as shown in **Table 1**. Under these conditions, the extraction was repeated three times.

Table 1. Orthogonal test of polysaccharides of Physalis alkekengi L. peel

No.	Material-to-liquid ratio [m(g):V(mL)]	Extraction temperature (℃)	Extraction time (min)	Yield (mg/g)
1	1:20	80	20	0.172
2	1:20	90	30	0.236
3	1:20	100	40	0.241
4	1:30	80	30	0.217
5	1:30	90	40	0.251
6	1:30	100	20	0.232
7	1:40	80	40	0.231
8	1:40	90	20	0.174
9	1:40	100	30	0.249
K1	0.216	0.207	0.193	
K2	0.233	0.219	0.235	
K3	0.218	0.241	0.239	
Polar deviation	0.017	0.034	0.046	
Optimal extraction process: 1:30, 100°C, 40 min, extraction influencing factors C > B > A.				

3.3. Verification of optimal extraction conditions for Physalis alkekengi L. polysaccharides

The optimal extraction process for *Physalis alkekengi L.* polysaccharides obtained from the orthogonal experiment was verified by repeating the extraction three times, confirming that this extraction process yields stable Physalis peruviana polysaccharides, as shown in **Figure 2**.

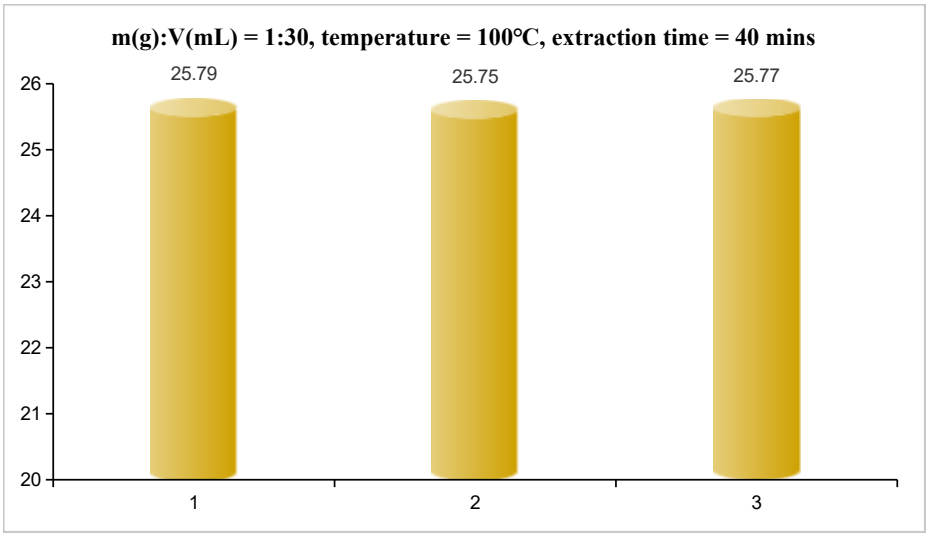

Figure 2. Verification of optimal extraction conditions for *Physalis alkekengi L.* polysaccharides

3.4. Antioxidant activity of Physalis alkekengi L. polysaccharides

The results of this study showed that the antioxidant activity of *Physalis alkekengi L.* polysaccharides was lower than that of ascorbic acid. However, as the concentration increased, the ability to scavenge DPPH, superoxide anion, and hydroxyl radicals also gradually increased, with scavenging rates reaching 68.67%, 61.46%, and 53.92%, respectively, as shown in **Figure 3**.

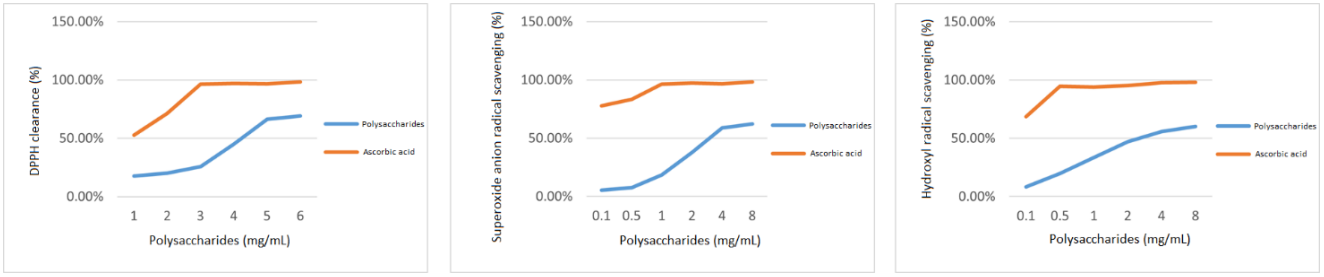

Figure 3. Antioxidant capacity of polysaccharides of *Physalis alkekengi L.*. **(left)** DPPH clearance (%); **(middle)** Superoxide anion radical scavenging (%); **(right)** Hydroxyl radical scavenging (%)

3.5. In vitro cytotoxicity experiment of Physalis alkekengi L. polysaccharides

The CCK8 assay was used to evaluate the growth inhibition and cytotoxicity of *Physalis alkekengi L.* polysaccharides on SW620 and HEK293 cells. The results showed that the cell viability of the SW620 group treated with 50 μg/mL, 100 μg/mL, and 200 μg/mL Physalis peruviana polysaccharides was significantly reduced ($P < 0.01$), while the HEK293 group treated with the same concentrations showed no cytotoxicity ($P > 0.05$), as shown in **Figure 4**. The IC_{50} value for *Physalis alkekengi L.* polysaccharides on SW620 cells after 24 hours was determined to be 150 μg/mL.

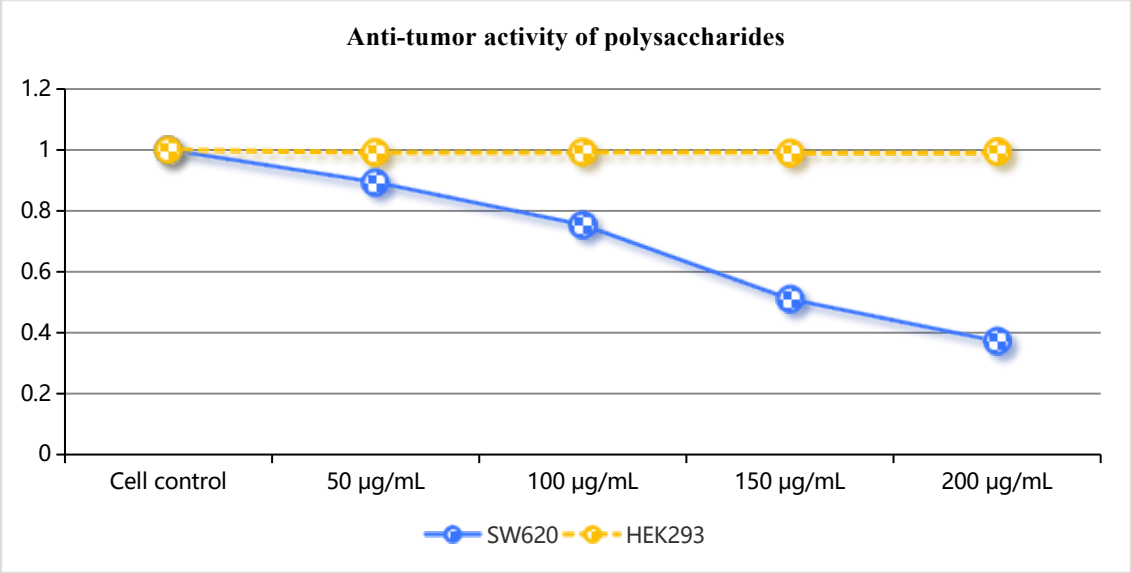

Figure 4. Cytotoxicity experiment of *Physalis alkekengi L.* polysaccharides on HEK293 cells and colorectal cancer cells SW620 showing growth inhibition of SW620 cells

3.6. Effect of Physalis alkekengi L. polysaccharides on the expression of inflammatory factors in SW620 cells

Compared with the SW620 control group, the relative expression level of p-NF-κB protein in the *Physalis alkekengi L.* polysaccharide-treated group was significantly reduced ($P < 0.01$), and the levels of IL-1β, IL-18, and TNF-α in the cell culture medium were also significantly decreased ($P < 0.01$), as shown in **Figure 5**, **Table 2**, and **Table 3**.

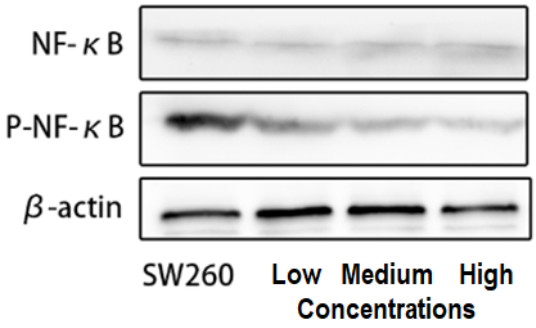

Figure 5. Western blot detection of NF-κB, p-NF-κB, and β-actin proteins

Table 2. Comparison of p-NF-κB protein expression levels in cells of each group

Groups	Relative expression of p-NF-κB protein
SW620	0.894 ± 0.09
Low concentration	0.334 ± 0.04
Medium concentration	0.256 ± 0.03
High concentration	0.126 ± 0.03*

*Comparison with the control group, $P < 0.01$.

Table 3. Comparison of levels of IL-1β, IL-18, and TNF-α in cell cultures of each group (pg/mL)

Groups	IL-1β	IL-18	TNF-α
SW620	126.305 ± 13.02	88.253 ± 9.12	283.259 ± 30.82
Low concentration	72.286 ± 4.52*	65.785 ± 2.76*	174.311 ± 16.23*
Medium concentration	41.412 ± 19.26	34.481 ± 1.55	95.571 ± 8.95
High concentration	15.131 ± 0.27†	10.026 ± 1.07†	62.506 ± 6.18†

*Comparison with the control group, $P < 0.01$; †Comparison with the low concentration group, $P < 0.01$.

4. Discussion

Colorectal cancer is one of the three most prevalent malignant tumors worldwide. The currently recommended primary treatments are surgery, radiotherapy, and chemotherapy, all of which are often accompanied by poor prognosis and high recurrence rates. The 5-year relative survival rate for colorectal cancer is approximately 65% [2]. The incidence and mortality rates of colorectal cancer are increasing every year, especially in large and medium-sized cities, making it a significant public health issue in China. However, one of the major challenges in treating colorectal cancer is the development of drug resistance. Cancer cells can develop resistance to chemotherapeutic drugs, thereby reducing treatment efficacy. Researchers are focusing on exploring efficient and non-toxic natural drugs. Based on research reports from 89 related literature sources, from 2018 to 2021, experts extracted 48 different types of polysaccharides with CRC inhibitory effects from various plants, including *Dendrobium officinale*, *Nostoc flagelliforme*, and *Ganoderma lucidum*, among others [5-7]. In this study, the extraction process of *Physalis alkekengi L.* peel polysaccharides was optimized using an orthogonal experiment based on a single-factor experiment. The final optimal aqueous extraction and alcohol precipitation process for *Physalis alkekengi L.* peel polysaccharides was determined as a material-to-liquid ratio of m(g):V(mL) = 1:30, extraction temperature of 100°C, and extraction time of 40 minutes, yielding a polysaccharide extraction rate of 25.7%.

The development of CRC may be due to the combined effects of inflammation and immune regulation. As a driver of intestinal inflammation, intestinal barrier dysfunction plays a critical role in the inflammatory mechanisms of CRC pathogenesis. Impaired intestinal barrier integrity allows bacteria-derived molecules and other antigens to cross the intestinal barrier to sustain this intestinal inflammation. Within the intestine, it can aid the inflammatory response by activating the NF-κB pathway through TLR4 receptors [8]. This major inflammatory pathway not only exacerbates intestinal barrier dysfunction but may also lead to the occurrence and progression of CRC [9].

Tumor necrosis factor-α (TNF-α) is a pleiotropic cytokine that primarily acts through the type 1 tumor necrosis factor receptor (TNFR1). After TNF binding, TNFR1 recruits TRADD (TNF receptor type 1-associated death domain). This interaction triggers the formation of signaling complexes claimed to induce apoptosis (through downstream caspase activation), inflammation (via NF-κB), and stress pathways (JNK/p38) [10].

It is important to emphasize that IL-1β, IL-18, and TNF-α are directly involved in cancer development [11]. For example, TNF-α stimulates tumor progression by activating NF-kB, thereby regulating processes such as invasion, migration, cell proliferation, inhibition of apoptosis, and tumor angiogenesis [12]. IL-18 can initiate tumorigenesis and/or tumor progression by inhibiting dendritic cell differentiation, inducing immune tolerance early in tumor development, and promoting metastasis [13]. Additionally, saliva samples from oral cancer patients have higher levels of IL-18 compared to healthy individuals or patients with precancerous lesions [14,15].

In conclusion, this study used an orthogonal experiment to determine the extraction process of Physalis peruviana peel polysaccharides and verified their antioxidant activity through *in vitro* experiments. Based on the research results, it can be preliminarily determined that *Physalis alkekengi L.* peel polysaccharides have anti-inflammatory effects, providing a reference for future CRC drug research.

Disclosure statement

The authors declare no conflict of interest.

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