Study on the Antitumor Effects of Crude Polysaccharides from Bamboo Fungi in Guizhou and Anhui Provinces

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Abstract: This study aimed to compare the inhibitory effects of crude polysaccharides extracted from Dictyophora indusiata sourced from Guizhou and Anhui provinces on tumor cells in vitro and their stimulatory effects on cancer–associated fibroblasts (CAFs) to evaluate the antitumor efficacy of these polysaccharides. Tumor cell growth was assessed using CCK and MTT assays as well as a real–time label–free cell analysis system. The cytotoxicity of crude polysaccharides on tumor cells was examined using flow cytometry, and the inhibitory effects on CAF growth were preliminarily evaluated. The results revealed regional differences in the antitumor effects of crude polysaccharides from D. indusiata and demonstrated that these polysaccharides could inhibit the proliferation of CAFs.

Keywords: Crude polysaccharides from Dictyophora indusiata; Polysaccharides with antitumor activity; Cancer–associated fibroblasts (CAFs)

Introduction

Dictyophora indusiata, a valuable edible and medicinal fungus, was first referenced in the Qing Dynasty by Xue Baochen in A Brief Discussion on Vegetarian Food. Wild D. indusiata primarily grows in the humus-rich soil beneath bamboo forests in central (western) Guizhou, Yunnan, Sichuan, Anhui, and other provinces of China [1,2]. Currently, D. indusiata can be cultivated on a large scale, with Guizhou's Zhijin County recognized as the "Hometown of D. indusiata" by the China Edible Fungi Association in 2000, while Qianshan County in Anhui Province is not a major production area. D. indusiata is known for its rich nutritional content, distinctive aroma, and delicious taste.

Recent studies have shown that many polysaccharides and polysaccharide complexes derived from fungi possess antitumor properties [3]. Specifically, crude polysaccharides from D. indusiata have been found to promote lymphocyte proliferation [4,5] and inhibit the growth of S180 sarcoma in mice [6], suggesting not only its nutritional value but also its potential disease-preventing and health-promoting effects. Polysaccharides are a key component of D. indusiata, and their physiological activities and functions have been widely reported [7–10]. However, to date, no studies have investigated the effects of D. indusiata crude polysaccharides on cancer-associated fibroblasts (CAFs) or compared the influence of different

production regions on their antitumor efficacy.

1 Materials and Methods

1.1 Materials and Equipment

1.1.1 Experimental Materials and Reagents

1. Dictyophora indusiata from Zhijin County, Guizhou Province, and Qianshan County, Anhui Province.
2. Polysaccharides from Lentinula edodes.
3. LLC (Lewis lung carcinoma) cells.
4. J558 mouse myeloma cells.
5. CT26 mouse colon carcinoma cells.
6. DMEM+/+ medium.
7. Phosphate-buffered saline (PBS).
8. Triplet reagent.
9. CCK-8 (WST) cell viability assay kit.
10. 5-Fluorouracil (5-FU).
11. Flow cytometry fluorescent dyes.

1.1.2 Equipment

1. Olympus optical microscope.
2. Cell counting chamber.
3. HERMLE centrifuge.
4. RT-CES real-time cell analysis system (RTCA).
5. Enzyme-linked immunosorbent assay (ELISA) reader.
6. BD flow cytometer.

1.2 Methods

1.2.1 Preparation of PBS and Triplet Reagents

To prepare 1× PBS, dissolve 8 g NaCl, 0.2 g KCl, 3.63 g $Na_2HPO_4 \cdot 12H_2O$, and 0.24 g KH_2PO_4 in 1000 mL ultrapure water.

To prepare the triplet reagent, dissolve 100 g 10% SDS, 50 mL 5% isopropanol, and 1 mL HCl in 1000 mL ultrapure water.

1.2.2 Extraction of Crude Polysaccharides from D. indusiata

Weigh 50 g of D. indusiata from Guizhou or Anhui, and add 1 L distilled water at a ratio of 1 g:20 mL. Boil at 80°C for 2 hours. After cooling, filter and wrap the residues in gauze to squeeze out the liquid. Add anhydrous ethanol to the filtrate at a 1:2 ratio, stirring with a glass rod to collect the white precipitate adhering to the rod. Transfer the precipitate and remaining liquid into centrifuge tubes. Centrifuge at 10,000 rpm for 5 minutes, discard the supernatant, and dry the precipitate to obtain crude polysaccharides.

1.2.3 MTT Assay for Tumor Cells Treated with Crude Polysaccharides

Seed 1×10^4 tumor cells per well in a 96-well plate. Add D. indusiata crude polysaccharides (from Guizhou or Anhui) or L.

edodes polysaccharides at 0.5, 1.0, 1.5, or 2.0 mg/mL. Add equal volumes of DMEM+/+ medium to control wells to reach a final volume of 1 mL per well. Add 150 μL PBS to edge wells. Incubate at 37°C for 24 hours. Add 20 μL MTT reagent to each well in the dark and incubate for an additional 4 hours. Carefully remove the supernatant, add 150 μL triplet reagent to each well, and shake for 10 minutes to dissolve the formazan crystals. Measure absorbance at 570 nm using an ELISA reader to calculate OD values.

1.2.4 Real-Time Cell Analysis of Tumor Cells Treated with Crude Polysaccharides

Seed 1×10^4 tumor cells per well in a 96-well plate. Add crude polysaccharides (Guizhou or Anhui) at 0.5, 1.0, 1.5, 2.0, or 2.5 mg/mL. Use 5-FU at 2 mg/mL as a positive control and DMEM+/+ medium as a negative control. Add 150 μL PBS to edge wells. Incubate at 37°C for 48 hours. Use the RT-CES real-time cell analysis system (RTCA, Roche Diagnostics, Shanghai) to monitor tumor cell growth inhibition through changes in impedance, reflecting cell morphology, adhesion, and viability in real time.

1.2.5 Flow Cytometry for Tumor Cells Treated with Crude Polysaccharides

Seed 5×10^4 cells per well in a 24-well plate. Add D. indusiata polysaccharides (Guizhou or Anhui) or L. edodes polysaccharides at 1.0 mg/mL. Use DMEM+/+ medium for negative controls and single-stain wells for comparison. Add 150 μL PBS to edge wells and incubate at 37°C for 24 hours. Collect supernatants into centrifuge tubes, wash cells with 200 μL PBS, and transfer suspensions into corresponding tubes. Count cells using a hemocytometer, centrifuge at 40,000 rpm and 4°C for 5 minutes, and resuspend the pellet in 50 μL PBS containing 2% NCS. Stain with PI dye at a 1:500 ratio for 30 minutes, centrifuge again, and resuspend in PBS with NCS. Transfer the cell suspension to flow cytometry tubes for analysis.

1.2.6 Statistical Analysis

Data were analyzed using GraphPad. Two-group comparisons were conducted with t-tests, and multiple-group comparisons were performed with ANOVA. Homogeneity of variance was tested, Levene statistics were calculated, and multiple group comparisons were conducted. The significance level was set at $p=0.05$.

2 Results and Analysis

2.1 Inhibition of Tumor Cells by Crude Polysaccharides from D. indusiata from Guizhou and Anhui

2.1.1 Morphological and Quantitative Observations of LLC Cells Treated with Crude Polysaccharides

LLC cells (1×10^4 cells/well) were seeded in 96-well plates and treated with 1.0 mg/mL crude polysaccharides from D. indusiata (Guizhou and Anhui) or Lentinula edodes for 48 hours in vitro. The effects on tumor cell growth were observed, and the results are presented in Figures 1 and 2 ($n=5$, $p<0.05$).

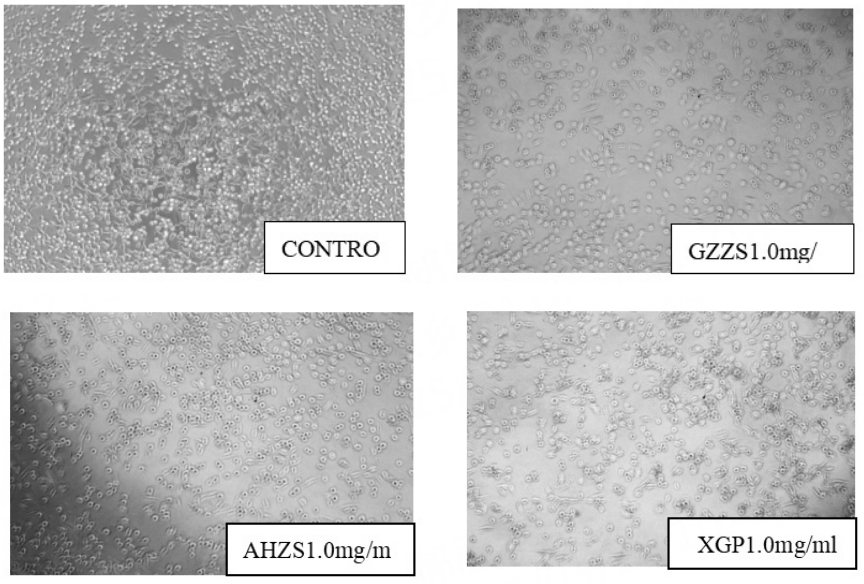

Fig. 1 Microscopic observation of LLC cells.

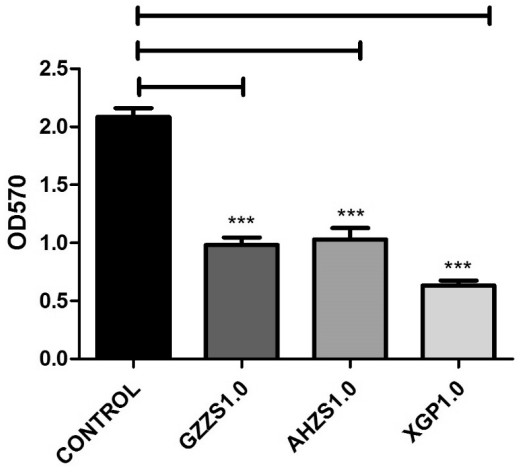

Fig. 2 Effects of Crude Polysaccharides from D. indusiata of Different Origins on Inhibiting LLC Cell Growth.

This experiment demonstrated that crude polysaccharides from D. indusiata from both regions exhibited inhibitory effects on LLC cells. The crude polysaccharides from Guizhou showed slightly stronger effects compared to those from Anhui, though their efficacy was inferior to that of Lentinula edodes polysaccharides. To further explore the antitumor effects of crude polysaccharides from D. indusiata of different origins, subsequent studies will validate these findings from multiple perspectives.

2.1.2 xCELLigence Analysis of LLC Cells Treated with Crude Polysaccharides from D. indusiata

LLC cells (1×10^4 cells/well) were seeded in 96-well plates and treated with crude polysaccharides from D. indusiata (Guizhou and Anhui) at concentrations of 0.5 mg/mL, 1.0 mg/mL, and 1.5 mg/mL for 48 hours in vitro. The results are shown in Figures 3 and 4.

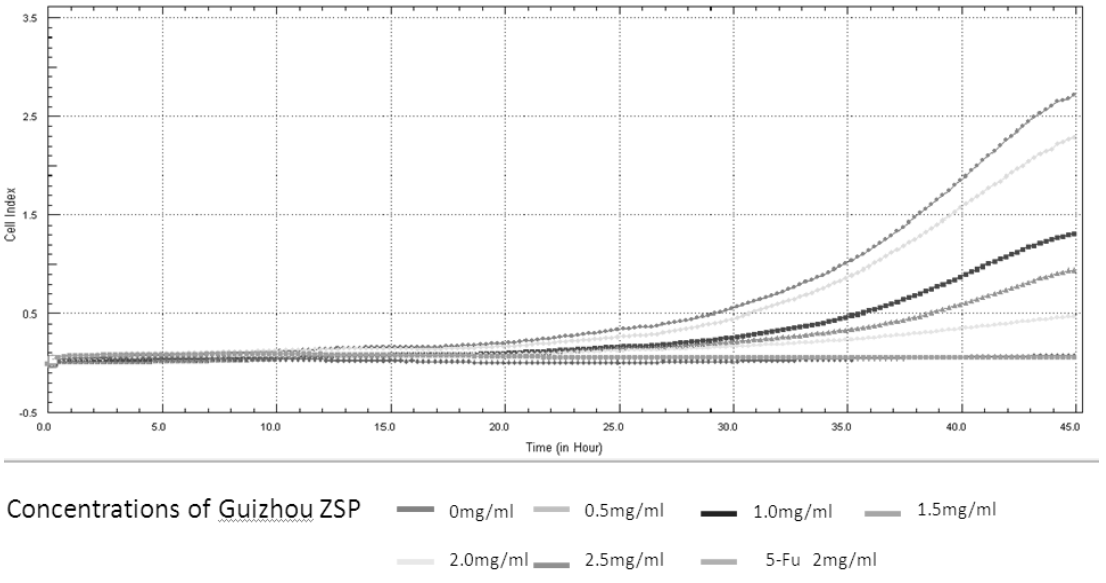

Fig. 3 Real–time Monitoring Curves of LLC Cell Growth Treated with Various Concentrations of Guizhou D. indusiata Crude Polysaccharides and 5–Fluorouracil.

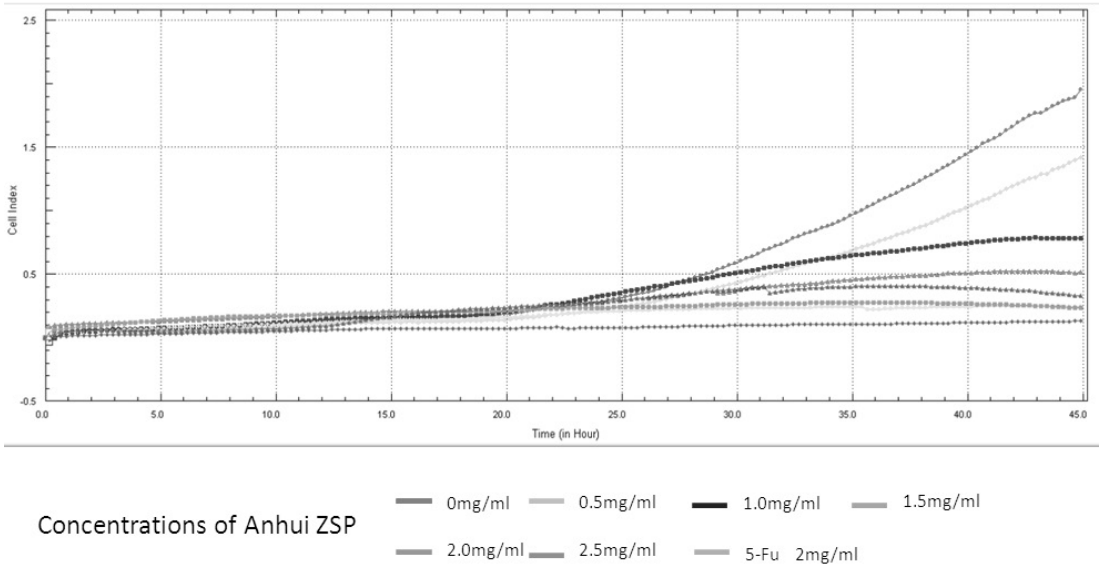

Fig. 4 Real–time Monitoring Curves of LLC Cell Growth Treated with Various Concentrations of Anhui D. indusiata Crude Polysaccharides and 5–Fluorouracil.

2.2 Stimulatory Effects of Guizhou D. indusiata Crude Polysaccharides on J558 and CT26 Cells In Vitro

J558 and CT26 cells (1×10^4 cells/well) were seeded in 96-well plates and treated with Guizhou D. indusiata crude polysaccharides at concentrations of 0.5, 1.0, 1.5, and 2.0 mg/mL for 48 hours in vitro. The results are shown in Figures 5 and 6 ($n=5$, $p<0.05$).

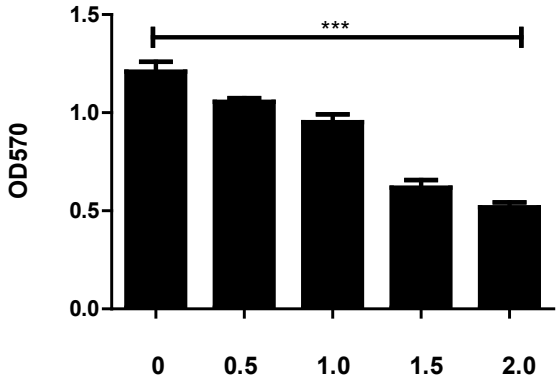

Fig. 5 Inhibitory Effects of D. indusiata Crude Polysaccharides on J558 Cell Growth.

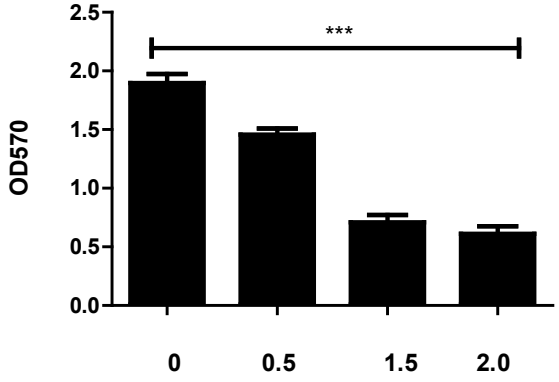

Fig. 6 Inhibitory Effects of D. indusiata Crude Polysaccharides on CT26 Cell Growth.

The experiments demonstrated that higher concentrations of D. indusiata crude polysaccharides resulted in more pronounced inhibitory effects on both J558 and CT26 murine tumor cells. These findings confirm that D. indusiata crude polysaccharides exhibit significant antitumor effects.

2.3 In Vitro Stimulatory Effects of Guizhou D. indusiata Crude Polysaccharides on J558-CAF Cells

J558-CAF cells (1×10^4 cells/well) were seeded in 96-well plates and treated with D. indusiata crude polysaccharides at concentrations of 0.5, 1.0, 1.5, and 2.0 mg/mL for 48 hours in vitro. The results are presented in Figure 7 ($n=5$, $p<0.05$).

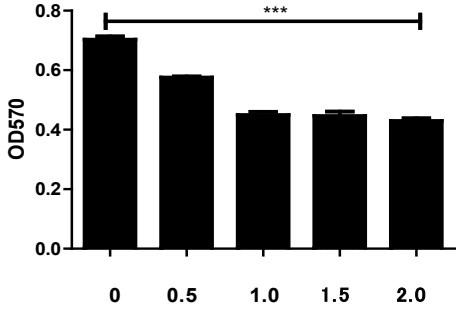

Fig. 7 Inhibitory Effects of D. indusiata Crude Polysaccharides on J558−CAF Cell Growth.

This experiment demonstrated that the number of J558 cancer-associated fibroblasts (CAFs) decreased with increasing concentrations of D. indusiata crude polysaccharides. The effects observed for the 1.0 mg/mL, 1.5 mg/mL, and 2.0 mg/mL groups were comparable. These findings suggest that D. indusiata crude polysaccharides exhibit significant inhibitory effects on CAFs in the tumor microenvironment.

3 Discussion

3.1 Analysis and Discussion

Cancer remains one of the most challenging diseases to treat clinically, posing a severe threat to human health. While current clinical approaches, such as chemotherapy and radiotherapy, provide hope for survival, they also cause considerable trauma to patients. Over the years, researchers have sought anticancer agents with fewer side effects, leading to a growing interest in polysaccharide-based antitumor therapies. Studies have shown that D. indusiata crude polysaccharides exhibit various biological activities, including tumor inhibition, immune modulation, lipid regulation, and antibacterial properties [11]. These findings highlight the significant potential of D. indusiata crude polysaccharides for antitumor research.

As a metabolic product of D. indusiata, its polysaccharides are not uniformly distributed across the plant in terms of quantity, quality, time, or space. Factors such as plant organs, tissues, cell types, growth stages, seasons, and environmental conditions influence polysaccharide content. As a perennial plant, D. indusiata exhibits variability in antitumor efficacy due to natural selection or artificial cultivation, even within individuals from the same region. Furthermore, the distribution and content of polysaccharides within different parts of the plant vary across developmental stages. Therefore, to maximize the antitumor efficacy of D. indusiata crude polysaccharides, it is essential to consider plant growth patterns and ecological factors, harvesting during peak polysaccharide accumulation.

Our study confirms that crude polysaccharides extracted from D. indusiata grown in Guizhou (a major production region) and Anhui (a secondary production region) significantly inhibit tumor cell growth. No significant differences in antitumor efficacy were observed between the samples from Zhijin County (Guizhou) and Qianshan County (Anhui) used in this study, indicating that the production region has minimal impact on their antitumor effects.

Under physiological conditions, the physicochemical properties of the cellular microenvironment are relatively stable. However, disruptions in microenvironmental homeostasis can lead to various pathological changes. The induction and maintenance of an abnormal extracellular microenvironment are considered critical in tumor formation and progression, making it a research hotspot in the field of cancer therapy. The tumor microenvironment, which includes tumor cells, cancer-associated fibroblasts (CAFs), and other cell types, serves as the internal environment for tumor cell growth and proliferation. Investigating the effects of D. indusiata crude polysaccharides on CAFs within the tumor microenvironment is therefore of significant importance.

This study demonstrates that D. indusiata crude polysaccharides have a notable inhibitory effect on J558 murine myeloma CAFs, suggesting their potential in modulating the tumor microenvironment.

3.2 Outlook and Applications

China is a primary producer of D. indusiata and was the first country to achieve artificial cultivation of this fungus. To ensure the continued rapid development of D. indusiata cultivation, relevant authorities should enhance the management of wild D. indusiata resources, prioritize the development of cultivation substrates, and focus on increasing yield per unit area. Research findings should be swiftly disseminated to producers. Additionally, there should be an emphasis on developing D. indusiata-

based products, including flavored foods, seasonings, nutritional health foods (beverages), and tonics, to further enhance the economic value of D. indusiata cultivation.

4 Conclusion

This study investigated the antitumor effects of D. indusiata from Guizhou and Anhui provinces, along with a preliminary exploration of its impact on the tumor microenvironment. The key findings are as follows:

1.The antitumor effects of D. indusiata from different regions are comparable.

2.D. indusiata crude polysaccharides significantly inhibit the growth of LLC, J558, and MCA205 cells.

3.D. indusiata crude polysaccharides exhibit modulatory effects on the tumor microenvironment.

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