

A Comparison Study of the Anticancerous Activity and Mechanism of Ethanolic Extracts from Different *Ganoderma lucidum* (W.Curt.:Fr.) Lloyd Strains

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Ganoderma lucidum (Ling Zhi or Reishi), a traditional Chinese medicine, has been used to promote health and prolong the life span in China for thousands of years. The evidence shows that triterpenes are the main effective components of *G. lucidum*. Some triterpenes extracted from *G. lucidum* fruiting body can effectively inhibit the proliferation of tumor cells in vitro, such as human hepatoma cells, cervix uteri tumor cells, breast cancer cells, and promyelocytic leukemia cells.

Compared to the cultivation of *G. lucidum* fruiting body, the liquid fermentation technique can achieve an industrial scale production with rigidly controlled manufacture conditions. It may be the future for producing high-quality *Ganoderma* products. However, there has not been much research addressed to the antitumor activity of triterpenes from mycelia. Only a few showed that triterpenes from mycelia could inhibit the proliferation of human hepatoma cells.

In this study, we used liquid fermentation to culture eight different *G. lucidum* strains and screened the strains producing high-anticancerous activity triterpenes against human acute promyelocytic leukemia HL-60 cell line.

It is known that triterpenes can be extracted with organic solvents such as ethanol, methanol, and chloroform. Therefore, we used ethanol to do the extraction. After that, a colorimetric method was used to detect the total triterpene contents of the extracts. MTT assay was applied to compare the

antiproliferation effects. Morphological observation, flow cytometric cell cycle analysis, and Annexin-V/PI bivariate assay were employed to identify the mechanism of antitumor activity.

The results showed that mycelia from all eight strains possessed inhibition effects against HL-60 proliferation. Among eight strains, ethanolic extract from strain L5 was identified to have the highest anticancerous activity. Its percentage of inhibition was 91.4 0.9% at a concentration of 125 μ /mL (72 hours). At the 48th hour, 13.3% of cells underwent early apoptosis after treatment. Cell cycle analysis revealed that in comparison with the control, the percentage of G₀/G₁ cells were elevated 15.9%, whereas that of S cells decreased 8.4% and G₂/M cells decreased 7.6%. It is the first time that the antitumor effect of *G. lucidum* mycelial triterpene containing extract was related to the cycle arrest in HL-60 cells. The results also clearly demonstrate that in addition to *G. lucidum* fruiting body, the mycelia can also produce anticancerous substances. *G. lucidum* could be the resource to provide candidates for antitumor substance screening.

Finally, the statistics showed total triterpene content did not have a close relation to the antitumor effect. Statistical results revealed that the total content can influence the antitumor activity, but the exact influence has not been elucidated. For example, the total triterpene content of L5 is 12.9%, just close to the mean of eight strains, but its percent of inhibition is as high as 91.4%, much higher than

the rest. This might be due to the content of the particular anticancerous triterpene(s) not being able to be detected when performing the total triterpene measurement. Therefore, it is necessary to detect the antitumor activity in addition to the total triterpene contents before the anticancerous triterpene(s) is identified and becomes measurable.

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