# IMPROVED AND COST EFFECTIVE METHOD FOR THE PRODUCTION OF *GANODERMA POLYSACCHARIDES*

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## Introduction

*Ganoderma lucidum* has always been regarded as a high quality herbal medicine for decades. one of the active biological compounds attributing to its medicinal values was found to be polysaccharides (Willard, 1990). Our previous studies had shown that there were many advantages in producing the products from the fungal mycelium instead of its fruiting bodies which had been used traditionally (Tong et al. 1994a,b). The objectives of this Project were: (a) to determine the nutritional requirements for optimum production of *Ganoderma* mycelium in liquid and solid medium; (b) to characterise the active polysaccharides that would promote the therapeutic properties; and (c) to establish potential beneficial qualities of locally grown *Ganoderma*, selected for their use as traditional nutrients, and/or medicinal properties, in providing protection against diseases and therefore improved in general health.

## Materials and Methods

Growing of fungal mycelium: *Ganoderma* mycelium was grown in both the liquid and solid medium using specially formulated composition in bottles and polystyrene bags, respectively. Extraction and purification of polysaccharides according to the method by Yang et al. 1994. Screening of bioactive compounds for anticancer or cytotoxic activity: The extract were tested against HeLa cell line (cervical carcinoma) where the IC5, or inhibition dose at 50 % will be determined. Antiviral activity: Screened against Herpes simplex-1 (DNA virus) and *vesticular stomatitis* virus (RNA virus) by using a modified plaque reduction assay. Antimicrobial property: Determined by using disc diffusion method and minimum inhibitory concentrator (MIC) against gram - ve. gram +ve, yeast and fungi of pathogenic strains.

## Results and Discussion

Three different species of *Ganoderma, namely G. lucidum, G. tsugae and G. tropicum* were selected for this study. The suitability of three liquid media, namely Media 1, 2 and 3 were tested for the cultivation of these cultures. The use of solid media for the same purpose is presently under investigation. A total of 24 carbon sources were tested for the optimal growth of *G. lucidum* mycelium in Medium 1. Dextrin, cellobiose, glycogen, starch and sucrose were found to be good carbon sources in enhancing the mycelial growth whereas rhamnose, pectic acid, inulin, cellulose, lignin, ribose and xylose only supported sparse growth. of the 25 nitrogen sources tested, L-phenylalanine, L-glutamine, L-arginine, L-valine and L-asparagine were the best organic nitrogen sources which yielded optimal growth of the mycelium. In the case of inorganic nitrogen, ammonium nitrate, ammonium chloride and sodium nitrate produced good mycelial growth. Overall, the growth was better with the organic nitrogen. Further supplementation with vitamins such as thiamine-HCI at concentration of 1.0 ug/g, riboflavin at 10.0 ug/g, nicotinic acid at 50 ug/g and inositol at 1.0 ug/g resulted significantly in a much luxurious growth of the mycelium. The best medium for the growth of the *Ganoderma* as well as the incubation period for optimal production of the mycelium was determined in each case. It was found that Medium 3 supported excellent growth of the three species of *Ganoderma.* The yield of mycelium for *G. lucidum* in Medium 3 was recorded as 1.8 g (d.w) compared to 0.6 g in Medium 2. For *G. tsugae,* a five (5) times dilution of the Medium 3 yielded maximum growth after an incubation of only 10 days compared to Medium 1 which took more than 20 days.

In terms of total polysaccharides produced, an optimal amount of the polysaccharides was obtained after an incubation period of about 20 days in Medium 3. More than 50 % of the polysaccharides produced were extracellular. Attention was drawn to the intracellular polysaccharides which were separated and purified by gel filtration. Their molecular weights together with their linkage types were determined. Water soluble extracts yielded negative results in the antimicrobial tests but further extraction using alcohol revealed positive response in inhibiting a number of bacteria. Particular attention is being focused on the β-1,3 and β-1,6 polysaccharides which possess therapeutic activities. Bioassays are presently been conducted on the crude extract to test for the anti-cholesterol, anti-tumour, anti-diabetes as well as anti-hypertension properties. Extracts identified to have potent activities in the test systems will be candidates for further studies to determine the physiological and pharmacological effects in vivo systems and also to determine the nature of the active constituents.

## Conclusions

*Ganoderma*, one of the riches source of health providing ingredients can be easily grown in Malaysia. As these active compounds are isolated from natural sources, extracts which demonstrate positive actions could easily be evaluated for potential use as functional food ingredients - either by incorporating into existing foods or by formulating new products by the food and allied industries. It is hope that this study would be able to help in achieving this goal in Malaysia.

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