

UNIVERSITI TEKNOLOGI MARA

**CYTOTOXICITY AND
GENOTOXICITY STUDIES OF
SELECTED MEDICINAL
MUSHROOMS EXTRACTS USING
CHINESE HAMSTER LUNG
FIBROBLAST CELL LINE (V79)**

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ABSTRACT

Medicinal mushrooms are well-known for nutritional benefits. However, it is necessary to evaluate the toxicity of medicinal mushrooms. The aim of this study was to identify the potential toxicity of *Agaricus blazei*, *Grifola frondosa* and *Hericium erinaceus*. In this study, screening of phytochemical compound, evaluating the cytotoxicity effect (MTT assay), determining the mode of cell death (Annexin V), observing the morphological changes of cell and analysing the genotoxicity (Comet Asssay) were done to assess the potential toxicity. All toxicity tests were evaluated against Chinese hamster lung fibroblast cell line (V79) using methanol and aqueous of the extracts at 0.125 mg/ml, 0.25 mg/ml, 0.5 mg/ml, 1 mg/ml and 2 mg/ml. Phytochemical screening showed the extracts contain abundantly of alkaloid. The MTT assay demonstrated that all extracts showed low cytotoxicity effect at 2 mg/ml. Only aqueous extract of *A. blazei* displayed the highest cytotoxicity effect at 1.7 mg/ml (IC_{50}). The Mode of Cell Death for viable V79 cells for *A. blazei* was higher at 2 mg/ml ($93.9\% \pm 0.721$) compared to apoptosis and necrosis using Annexin V. Cell morphology did not show any sign of apoptotic and cell injury at 1 mg/ml and 2 mg/ml of mushroom extract during 24 hours treatment. Lastly, genotoxicity activity for *A. blazei*, *G. frondosa* and *H. erinaceus* in V79 cell results revealed that DNA damages were detected at 1 mg/ml and 2 mg/ml. In conclusion, these three selected medicinal mushrooms had caused toxicity to normal cells as demonstrated by V79 cell line at 1 to 2 mg/ml.

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CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

In the era of globalization, medicine profession and the pharmaceutical field are growing rapidly along with various drug discoveries. Nowadays, a variety of drug discoveries in modern medicines were claimed can cure many diseases (Singh, 2010). In addition, Newman and Cragg (2012) have discovered natural products as sources of new drugs over the 30 years from 1981 to 2010.

World Health Organization (WHO) in the year of 2008 has estimated that approximately 80% of the people in developing countries still rely on traditional medicine as their primary health care. Tradition is the transmission of cultural beliefs from generation to generation and that are most valuable (Moe *et al.*, 2014). Approximately of 86% traditional medicines involved plant extracts (Cowan, 1999; Elgorashi *et al.*, 2003). Usually, mushroom, herbs and spices are always consumed for the treatment of traditional medicines (Saad, 2015; Shaw and Charters, 2016).

Medicinal mushrooms were chosen to further studies because previous studies mentioned about the effectiveness of the mushroom products as a good supplement for health (Neculita *et al.*, 2011; Gea *et al.*, 2014; Oloke and Adebayo, 2015). However, lack of toxicological study from the medicinal mushrooms has been reported. Further study on the consumption of mushroom must be carried out because mushroom has different ecological properties compared to other plants. Usually, mushrooms obtained the nutrients from dead organisms due to their parasitic and saprotrophic properties.

In this study, *Agaricus blazei*, *Grifola frondosa* and *Hericiium erinaceus* were studied because they are widely consumed in many countries including Malaysia. There are several companies in Malaysia which use those mushrooms in pharmaceutical and wellness products such as Ganofarm Sdn Bhd and Bioresis Sdn Bhd. Those mushrooms were cultivated in China due to suitable climate as compared in Malaysia. Zhang *et al.* (2014) and Rosmiza *et al.* (2016) have pointed out that China is the main exporters for medicinal mushrooms and market expansion of the mushroom business in Malaysia is growing rapidly. However, important toxicological