

**UNIVERSITI TEKNOLOGI MARA**

**CYTOTOXICITY OF METHANOL  
AND AQUEOUS *Lignosus rhinocerus*  
(Tiger Milk mushroom) EXTRACTS  
ON HCT 116 HUMAN COLORECTAL  
CANCER CELLS AND ITS MODE OF  
ACTION**

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Thesis submitted in the fulfilment  
of the requirements for the degree of  
**Master of Science**

**Faculty of Applied Sciences**

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## CONFIRMATION BY PANEL OF EXAMINERS

I certify that a Panel of Examiners has met on 7<sup>th</sup> January 2015 to conduct the final examination of Suziana Zaila Bt Che Fauzi on her Master of Science thesis entitle “Cytotoxicity of methanol and aqueous *Lignosus rhinoceros* (Tiger Milk Mushroom) extracts on HCT 116 human colorectal cancer cells and its mode of action” in accordance with Universiti Teknologi MARA Act 1976 (Akta 173). The Panel of Examiners recommends that the student be awarded the relevant degree. The panel of Examiners was as follow:

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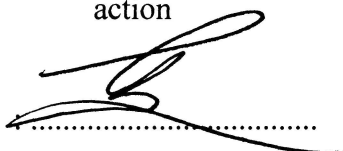
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I declare that the work in this thesis was carried out in accordance with the regulation of Universiti Teknologi MARA. It is original and is the result of my own work, unless otherwise indicated or acknowledged as referenced work. This topic has not been submitted to any other academic institution or non-academic institution for any other degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulation for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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## ABSTRACT

Cancer is a very dangerous disease and fear by many. The available clinical treatment of cancer as chemotherapy, radiotherapy and surgery was expensive, toxic that cause severe effects and follow the concept of one drug – one target action. In this study, natural product which is *Lignosus rhinocerus*, commonly known as Tiger Milk mushroom, belongs to Polyporaceae family was used because of their less toxicity and cheaper than synthetic drugs. The mushroom is traditionally used in Malaysia for many beneficial claims such as cough, asthma, fever, chronic hepatitis and cancer. The biological activity of *L. rhinocerus* was investigated in terms of its cytotoxic effects, mode of cell death and antioxidant capacity. The antiproliferative activity of methanolic and aqueous PLEs was determined using MTT cell viability assay, where  $IC_{50}$  value was used as a parameter for mode of cell death. MTT assay showed that both extracts significantly reduced the cell viability in a dose-dependent manner. Our results showed that methanol PLE of *Lignosus rhinocerus* sclerotia was found to exhibit antiproliferative activity against HCT 116 cells, with an  $IC_{50}$  of 600  $\mu\text{g/mL}$  and 1200  $\mu\text{g/mL}$  for aqueous PLE but not cytotoxic to normal human colon cell (CCD-18-Co) and fibroblast Chinese hamster lung cell (V79-4). The  $IC_{50}$  values suggested that the solvent differences in pressurized liquid extraction greatly affected their antiproliferative activities. The Tiger Milk mushroom PLE arrested HCT 116 cells at G2/M phases with corresponding decreased in S-phase. The types of cell death were explored by flow cytometry using the Annexin V/FITC and propidium iodide and the dead end fluorometric TUNEL System. The mode of cell death induced by *Lignosus rhinocerus* PLEs was primarily apoptosis by externalization of phosphatidylserine and DNA damage detected by 3'-OH DNA ends labelling. Additionally, weak antioxidant capacities were detected in both methanol and aqueous PLE of *L. rhinocerus* by the FRAP (Ferric Reducing Antioxidant Power) Assay. However, this extract did not increase the endogenous antioxidant capability when tested against HCT 116 cells. Preliminary phytochemical screening revealed that both methanol and aqueous pressurized liquid extraction of *L. rhinoceros* contained alkaloids. The above data suggested that pressurized liquid extraction (PLE) extract of the sclerotia of *L. rhinocerus* possesses cytotoxicity to human colorectal cancer cells but were non-toxic to the corresponding normal cells. The results suggest that Tiger Milk mushroom stimulates apoptosis through G2/M cell cycle arrest.

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