



UNIVERSITI PUTRA MALAYSIA

CYTOTOXIC EFFECT OF *CENTELLA ASIATICA* AND *PETROSELINUM CRISPUM* METHANOLIC EXTRACT ON CANCER CELL LINE

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FBSB 2015 102

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**Dissertation submitted in partial fulfillment of the requirement for the course
BMY 4999 Project in the Department of Microbiology
Universiti Putra Malaysia
JUNE 2015**

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2015

PENGESAHAN

Dengan ini adalah disahkan bahawa projek yang bertajuk “Cytotoxic effect of *Centella asiatica* and *Petroselinum crispum* methanolic extract on cancer cell line” telah disiapkan serta dikemukakan kepada Jabatan Mikrobiologi oleh Farah Munirah Binti Mohamed (163122) sebagai syarat untuk kursus BMY 4999 projek.

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ABSTRACT

A phenomenon which cells divide abnormally and able to spread to other parts of the body is known as cancer. It is a genetic disease, it changes the genes that regulate the cell functions. Conventional treatments available are expensive and the result is not promising. Thus, the demand in finding an alternative treatment with an affordable price is high. In order to produce an affordable treatment, natural products seem to be the best options. Among millions of plants available, *Centella asiatica* and *Petroselinum crispum* were chosen. These plants are traditionally used to treat various diseases including cancer. The study was done to determine the cytotoxic effect of *C. asiatica* and *P. crispum* methanolic extract on cancer cell line. To achieve this goal, the plant extracts were tested on Saos-2 cells. Both Saos-2 cells and Chang liver cells (used as control) were seeded on 96-well plates. 5% DMSO was used as the positive control of the experiment. Then the cells were subjected to treatment with the plant extract for 24, 48 and 72 hours. The cytotoxic effect of the cells was evaluated by using MTT assay. Formation of purple formazan was dissolved with 100 μ l DMSO and read at 570 nm with reference wavelength 690 nm. The data obtained were translated into graphical format and the IC₅₀ were determined. Extract of *C. asiatica* reacted strongly towards Saos-2 cells, however, it also showed adverse effects on normal cells (Chang liver cells). This makes it unsuitable for human consumption. Extract of *P. crispum* has a lower cytotoxic effect than *C. asiatica*, and this extract did not have an adverse effect on normal cells. Thus, *P. crispum* seems to be more promising candidates for cancer treatment.

ABSTRAK

Fenomena dimana sel membahagi secara tidak terkawal dan merebak ke seluruh badan dikenali sebagai kanser. Ia adalah sejenis penyakit genetik, ia menukar fungsi gen yang mengawal fungsi-fungsi sel. Rawatan konvensional yang sedia ada adalah mahal dan kesannya tidak memberangsangkan. Oleh itu, keperluan untuk mencari rawatan alternative dengan harga yang berpatutan adalah tinggi. Dalam usaha mencari rawatan alternative dengan harga yang murah, produk semula jadi adalah pilihan terbaik. Dalam banyak-banyak tumbuhan herba yang ada, *Centella asiatica* dan *Petroselinum crispum* dipilih untuk kajian ini. Tumbuhan ini digunakan secara tradisional untuk merawat pelbagai penyakit termasuk kanser. Kajian ini dilakukan untuk melihat kesan sitotoksik ekstrak metanol *C. asiatica* dan *P. crispum* pada sel kanser. Untuk mencapai matlamat ini, ekstrak metanol kedua-dua tumbuhan diuji pada sel Saos-2. Sel Saos-2 dan sel hati Chang (digunakan sebagai control) dipilih untuk diuji, kedua-dua sel di biarkan tumbuh pada plat telaga 96. 5% DMSO digunakan sebagai kawalan positif eksperimen. Kemudian kedua-dua sel dirawat dengan ekstrak metanol tumbuh-tumbuhan selama 24, 48 dan 72 jam. Kesan sitotoksik sel dinilai dengan menggunakan MTT assay. Pembentukan formazan ungu yang terhasil dilarutkan dengan menggunakan 100 μ l DMSO dan dibaca pada 570 nm dengan 690 nm sebagai rujukan. Data yang diperoleh diolah ke dalam format grafik dan IC_{50} juga diperoleh. Ekstrak *C. asiatica* memberi tindak balas sitotoksik yang sangat jelas pada sel Saos-2, tetapi ia juga menunjukkan kesan sitotoksik pada sel normal. Ini menunjukkan ekstrak ini tidak sesuai untuk kegunaan manusia. Ekstrak *P. crispum* mempunyai kesan sitotoksik lebih rendah daripada ekstrak *C. asiatica* dan ekstrak ini tidak menunjukkan kesan sitotoksik pada sel normal. Oleh itu, ekstrak *P. crispum* dilihat sebagai tumbuhan yang lebih sesuai untuk dijadikan sebagai ubat untuk merawat kanser.

ACKNOWLEDGEMENT

I am very glad and thankful to Allah S.W.T. for His blessing for me to finish this project successfully. This project would not have been possible without the support, guidance, assistance and encouragement of the following persons, and I wish to extend my deepest gratitude to each of them.

I would first like to thank my supervisor, Dr. Norazizah Shafee, for her assistance, guidance and great suggestions throughout the project. Thanks for making the project possible.

My sincere appreciation is extended to my parents and family for their continuous supports and encouragements. They even helped me collecting my sample for the project. I would also like to thank all postgraduate students in virology lab 143, especially Liew Sien Yei for the many days and nights that she had to help, guide and assist me throughout the project.

I would also use this opportunity to thank all friendly staff members of the faculty and classmates. Thanks for your time, support and encouraging words which had motivated me to complete this project. Thank you to everyone who has directly or indirectly helped in the completion of this project.

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LIST OF ABBREVIATIONS

%	Percentage
µl	Microliter
ml	Milimeter
mg	Milligram
°C	Degree of celcius
mg/ml	Milligram per mililiter
g	Gram
O.D.	Optical density
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide
ATCC	American type culture collection
IC ₅₀	Inhibition concentration 50%
DMEM	Dubelco's modified Eagle's Medium
DMSO	Dimethyl sulfoxide
Saos-2	Bone cancer cell line
PBS	Phosphate-buffered saline
FBS	Fetal bovine serum

CHAPTER 1

INTRODUCTION

One of the life-threatening health problems in the world now is cancer (Jemal et al., 2007). The current treatment or therapy available is surgical treatment, radiotherapy, chemotherapy, immunotherapy, phototherapy, hyperthermia therapy and combined treatment (Baba et al., 2007). However, conventional cancer therapies cause serious side effects, and merely extend the patient's lifespan by a few years (Amin et al., 2009). The most common side effects of cancer chemotherapy and radiotherapy includes nausea and vomiting (Schnell, 2003).

Other alternative cancer treatments available is complementary and alternative medicine (CAM) which is defined as a group of diverse medical and health care systems, therapies, and products that are not presently considered to be part of conventional medicine (Barnes et al., 2004). However, little is known about the use of CAM in cancer patients specifically (Molassiotis et al., 2005). Natural product is now considered as an emerging role as alternative and complementary medicines in cancer prevention, they offer promising strategies for chemoprevention in order to block the development of cancer in human (Farshori et al., 2013). Plant-derived compounds have been an important source of several clinically useful anti-cancer agents (Cragg & Newman, 2005). Through scientific research it is indicated that the bioactive components present in herbs and spices can reduce the risk of cancer through its antitumorogenic activity and its ability to directly suppress carcinogen bioactivation (Kaefer & Milner, 2008).

The purpose of the study is to find an alternative treatment for cancer using plant methanolic extract. The plant of interest in this study is *Centella asiatica* and *Petroselinum crispum*. The plant was chosen based on their common usage in Malaysia. *In vitro* cytotoxicity of extract towards cell was evaluated by using MTT assay (Liew et al., 2012 and Vijayarathna & Sasidharan, 2012). Hypothesis of this study is *C. asiatica* and *P. crispum* methanolic extract are able to inhibit cancer cell growth. In order to evaluate the cytotoxic effect of *C. asiatica* and *P. crispum*, three specific aims were designed. They are:

1. To prepare crude methanolic extract of *C. asiatica* and *P. crispum*.
2. To optimize cell density and diluent concentration.
3. To determine the cytotoxic effect of *C. asiatica* and *P. crispum* methanolic extract on the human osteosarcoma (Saos-2) cells.

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