UNIVERSITI PUTRA MALAYSIA

ANTIPROLIFERATIVE ACTIVITY OF METHANOLIC EXTRACTS OF GENUS *Allium* ON HUMAN OSTEOSARCOMA SAOS-2 CELLS

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PENGESAHAN

Dengan ini adalah disahkan bahawa projek yang bertajuk " Antiproliferative Activity of Methanolic Extracts of Genus *Allium* on Human Osteosarcoma Saos-2 cells " telah disiapkan serta dikemukakan kepada Jabatan Mikrobiologi oleh Cheow Pheik Sheen (161350) sebagai syarat untuk kursus BMY 4999 projek.

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ABSTRACT

Conventional treatments towards cancer bring along side effects to the patients. Plant extract treatments appear as new candidates for cancer treatment with fewer side effects. However, cytotoxicity effects of plant extracts on cancer are still lacking in scientific evidence. *Allium* plant extracts were hypothesed to able to inhibit growth of cancer cells. In this study, the cytotoxicity effects of two *Allium* plant extracts on Human osteosarcoma, Saos-2 cells were examined. Cell viability of Saos-2 cells was compared to the normal cells, Chang Liver Cells. *Allium sativum* fruit bodies and *Allium ampeloprasum* leaves were dried and blended into powder form. Then, they were extracted with methanol and evaporated to obtain crude extracts. Various concentrations of *Allium* extracts were used to treat the Human Saos-2 osteosarcoma cells. MTT assay was performed after 24, 48 and 72 hours of post treatment to evaluate the cytotoxicity effects. *Allium sativum* showed inducing of cell proliferation on Chang Liver Cells after 24h of treatment. In the other hand, it did not inhibit significant cell viability of Saos-2 cells for three different time slots. *Allium ampeloprasum* exhibit cytotoxicity towards both cell lines, but it kills more Chang Liver Cells than Saos-2 cells.

ABSTRAK

Rawatan konvensional kanser boleh membawa kesan sampingan kepada pesakit. Rawatan ekstrak tumbuh-tumbuhan telah muncul sebagai calon baru untuk rawatan kanser dengan kesan sampingan yang lebih minima. Walau bagaimanapun, kesan sitotoksiti ekstrak tumbuh-tumbuhan masih kekurangan bukit saintifik. Ekstrak tumbuh-tumbuhan mempunyai hypothesis dapat menghalang pertumbuhan sel-sel kanser. Dalam kajian ini, kesan sitotoksiti dua ekstrak tumbuhan Allium pada sel osteosarcoma manusia, Saos-2 sel telah dikaji. Pertumbuhan sel Saos-2 dibandingkan dengan sel biasa, Chang Liver sel. Buah Allium sativum dangan daun Allium ampeloprasum telah dikeringkan dan dikisarkan kepada serbuk. Kemudian, serbuk tersebut telah diekstrak dan disejat untuk mendapat ekstrak mentah. Pelbagai kepekatan ekstrak Allium telah digunakan untuk merawat sel osteosarcoma manusia, Saos-2 sel. MTT telah dilakukan selepas 24, 48 dan 72 jam untuk menilai kesan sitotoksiti kedua-dua tumbuh-tumbuhan. Allium sativum menunjukkan dalam mendorong dalam percambahan sel pada 24 jam rawatan. Di sisi lain, ia tidak menghalang percambahan sel osteosarcoma manusia, Saos-2 sel untuk tiga slot masa yang berbeza. Allium ampeloprasum menunjukkan sitotoksiti bagi kedua-dua sel, tetapi ia membunuh lebih sel biasa, Chang Liver sel daripada osteosarcoma manusia, Saos-2 sel.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figures **Page**

LIST OF ABBREVIATIONS

A. ampeloprasum	*Allium ampeloprasum*
A.sativum	*Allium sativum*
CAM	Complementary and alternative medicine
CLC	Chang liver cells
CO^2	Carbon dioxide
DMEM	Dulbecco's modified eagle medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
FBS	Fetal bovine serum
IC_{50}	Half maximal inhibitory concentration
MTT	3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide
MTS	4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium
NCCAM	National center for complementary and alternative Medicine
NCI	National cancer institute
SAMC	S-allymercaptocysteine
SEM	Standard error mean
^0C	Degree celcius
%	Percent

CHAPTER 1

INTRODUCTION

Cancer is a class of disease which is induced by the growth of abnormal cells and have the ability to invade other tissues (King, 2008). Cancer cells differ from normal cells as they are less specialized and they keep up a continuous growth by mitosis (Warburg, 1956). In year 2008, International Agency for Cancer Research had reported the discovery of 12.7 million new cancer cases and 7.6 million of death caused by cancer worldwide (Baliga & Dsouza, 2011). In Malaysia, cancer had overtaken heart disease as the number one killer medically reported death. In year 2007, the three leading cancer cases reported in Malaysia are breast cancer (18.1%), head and neck cancer (13.2%) and colorectal cancer (12.3%) (Cancer Research Initiatives Foundation, 2014).

Current clinical therapies include radiotherapy, chemotherapy and multi drug treatment or combinations of them. It is necessary to search for a novel therapies as the current clinical cancer treatment severe toxicity and drug resistance (Zhang et al., 2011).

Madhuri and Pandey (2008) indicated that traditional medicine is taken as an alternative path to treat cancer as they are capable to cure disease without causing any toxicity to the normal cells. Based on modern herbal medicine books, all these naturally occurring plant anticancer formulations belong to the complementary and alternative medicine (CAMs)

(Cheng et al., 2005). National Center for Complementary and Alternative Medicine (NCCAM) defines CAM as a group of diverse medical and health care practices, systems and products which is not a part of present conventional medicine (Yates et al., 2005). There were many plant extracts reported able to inhibit cancer cells (Ashidi et al., 2010; Kuete et al., 2011; Steenkamp & Gouws, 2006).

In this study, we hypothese that *Allium* methanolic plant extracts are able to exhibit anti-proliferation effects on cancer cell lines. Therefore, the main objective of the study is to evaluate cytotoxicity effect of *Allium* methanolic plant extracts on Human osteosarcoma cell line, Saos-2 cells. To achieve the objective, there are three specific objectives :

1. To prepare crude methanolic extracts of *Allium sativum* and *Allium ampeloprasum*
2. To optimize the optimal cell densities and diluent concentration for anti-proliferation assay
3. To evaluate cytotoxicity effect of *Allium sativum* and *Allium ampeloprasum* crude methanolic extracts on cancer cell line

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