ANTIPROLIFERATIVE EFFECT OF ACANTHACEAE SPECIES ON HUMAN BREAST CANCER CELL LINE MCF7

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KEVIN LARSON S GALONO

THIS DISSERTATION IS SUBMITTED AS PART OF THE REQUIREMENT TO OBTAIN THE BACHELOR OF SCIENCE DEGREE WITH HONOURS

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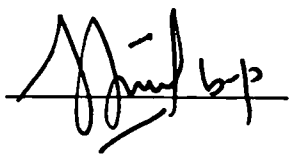

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ABSTRACT

This experiment was done in order to investigate the effectiveness of the extract from Acanthaceae species in inhibiting the proliferative of human breast cancer cell MCF7. Plant extracts were obtained from *Strobilanthes crispus* (SC), *Clinacanthus nutans* (CN), and *Ruellia tuberosa* (RT). Methanol leaf extract from SC (SCML) showed an IC_{50} value at 85 µg/ml while methanol stem extract (SCMS) showed an IC_{50} of 76 µg/ml. Ethyl acetate leaf extract from SC (SCEAL) showed IC_{50} value at 89 µg/ml while for ethyl acetate stem of SC (SCEAS) showed IC_{50} value at 83.5 µg/ml. Value of IC_{50} for SC is very high, meaning the low cytotoxic activity compared to other extracts. IC_{50} for methanol CN root (CNMR) extract was IC_{50} at 41 µg/ml while ethyl acetate for CN root showed the lowest IC_{50} at 8.5 µg/ml, meaning high level of cytotoxicity. Methanol of RT leaf extract showed the IC_{50} value at 37 µg/ml, while for stem IC_{50} showed at 34 µg/ml. IC_{50} for ethyl acetate of RT leaf extract (RTEAL) was 45 µg/ml, and for RTEAS showed at 34.5 µg/ml. The combination treatments were done to investigate the effectiveness of the treatment towards the human breast cancer cell line MCF7. For combination treatment that showed indifferent effect were the combination of SCEAS and RTEAL extracts at concentration ratio of 1:1 and 2:1, the combination of SCEAL and RTEAL at concentration ratio 1:1 and 2:1, and extract combination of SCEAS and RTML at ratio 1:1 and 1:2. The combination of SCEAS and RTEAL at the ratio 1:2 showed an effective additive effect with low Frictional Inhibitory Index (FIX) value. Antagonistic effect was found in cells treated with extract SCEAL and RTEAL at concentration ratio 1:2 and extract SCEAS and RTML at ratio 2:1. Cell morphology was studied in order to determine the morphological changes of the cell in the treatment. Some apoptotic-like features such as cell nucleus condensed, apoptotic body and ring formation in nuclear envelope were observed. Overall, cell treated with plant extract CNEAR shows the highest antiproliferative effect at the lowest IC_{50} value, while combination of SCEAS and RTEAL at the ratio of 1:2 is the best combination treatment because it shows additive effect.

ABSTRAK

Eksperimen ini bertujuan untuk mengkaji keberkesanan ekstrak tumbuhan spesis Acanthaceae dalam menghalang pertumbuhan sel kanser payu dara manusia MCF7. Ekstrak tumbuhan diambil dari tumbuhan *Strobilanthes crispus* (SC), *Clinacanthus nutans* (CN), dan *Ruellia tuberosa* (RT) pada bahagian tumbuhan yang berbeza. Ekstrak methanol daun SC (SCML) menunjukkan nilai IC_{50} 85 µg/ml manakala ekstrak metanol batang SC (SCMS) memberi nilai IC_{50} sebanyak 76 µg/ml. Etil asetat bagi daun SC (SCEAL) mencatatkan nilai IC_{50} tertinggi, sebanyak 89 µg/ml manakala pada batang SC (SCEAS) mencatatkan nilai IC_{50} sebanyak 83.5 µg/ml. Nilai IC_{50} bagi tumbuhan SC adalah sangat tinggi, menunjukkan aktiviti sitotoksik yang rendah berbanding tumbuhan lain. IC_{50} bagi metanol ekstrak akar CN (CNMR) dicatatkan pada 41 µg/ml manakala etil asetat pada akar CN (CNEAR) menunjukkan aktiviti sitotoksik yang tertinggi dengan nilai IC_{50} yang terrendah, 8.5 µg/ml. Metanol pada daun RT (RTML) menunjukkan nilai IC_{50} sebanyak 37 µg/ml, dan pada batang menunjukkan IC_{50} sebanyak 34µg/ml. IC_{50} bagi etil asetat pada daun RT (RTEAL) dicatatpada 45 µg/ml, dan pada batang RT sebanyak 34.5 µg/ml (RTEAS). Kajian rawatan kombinasi dijalankan bagi mengkaji keberkesanan rawatan gabungan ekstrak tersebut pada sel kanser payu dara manusia MCF7. Rawatan gabungan yang menunjukkan kesan gabungan yang tidak berbeza termasuklah gabungan ekstrak SCEAS dan RTEAL pada nisbah kepekatan 1:1 dan 2:1, gabungan ekstrak SCEAL dan RTEAL pada nisbah kepekatan 1:1 dan 2:1, dan gabungan ekstrak SCEAS dan RTML pada nisbah kepekatan 1:1 dan 1:2. Gabungan SCEAS dan RTEAL pada nisbah kepekatan 1:2 menunjukkan kesan aditif. Gabungan ini adalah sangat efektif kerana menunjukkan hasil aditif pada kadar "*Frictional Inhibitory* Index" (FIX) yang rendah. Kesan antagonistik dicatat dalam rawatan menggabungkan ekstrak SCEAL dan RTEAL pada nisbah kepekatan 1:2 dan ekstrak SCEAS dan RTML pada nisbah kepekatan 2:1. Morfologi sel juga dikaji bagi memastikan sel yang dirawat melalui fasa apoptosis. Ciri-ciri apoptotik sel yang ketara memperlihatkan kromosom nukleus sel yang padat, formasi badan apoptotik dan pembentukan bulatan pada envelop nuklear sel tersebut. Justeru, CNEAR menunjukkan ekstrak yang terbaik bagi mendapatkan kesan antiproliferasi pada kepekatan yang rendah, manakala rawatan paling efektif menggabungkan SCEAS dan RTEAL pada nisbah kepekatan 1:2 yang menunjukkan kesan aditif.

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DNA	Deoxyribonucleic Acid
Rb	Retinoblastoma
p53	Protein tumour
DMEM	Dulbecco's Modified Eagle Medium
ELISA	Enzyme-linked immunosorbent assay
MTT	Mossman's Tetrazole Test
PBS	Phosphate-buffered saline
Rpm	Rotation per minute
CO_2	Carbon dioxide
SCML	*Strobilanthes crispus,* methanol leaf extract
SCEAL	*Strobilanthes crispus,* ethyl acetateleaf extract
SCMS	*Strobilanthes crispus,* methanol stem extract
SCEAS	*Strobilanthes crispus,* ethyl acetate stem extract
CNMR	*Clinacanthus nutans,* methanol root extract
CNEAR	*Clinacanthus nutans,* ethyl acetateroot extract
RTML	*Ruellia tuberosa,* methanol leaf extract
RTEAL	*Ruellia tuberosa,* ethyl acetateleaf extract
RTMS	*Ruellia tuberosa,* methanolstem extract
RTEAS	*Ruellia tuberosa,* ethyl acetate stem extract

CHAPTER 1

INTRODUCTION

1.1 Introduction

Cancer is a disease caused by an abnormal proliferation of cell. It affects the health and can lead to major health issues, and even death. Cancer is the number one public health issue in the world. When a cell fails to achieve normal cell growth, the proliferation of cell will become abnormal and affects other cells and tissues in other parts of the body (Yang *et al.*, 2013). Categories of cancer includes carcinoma (formed from the skin or tissue), sarcoma (formed from connective tissue or cartilage and muscles), leukemia (formed from the blood forming tissue which causes excretion of abnormal red blood cell that enters through blood capillaries), lymphoma and myeloma that starts in the immune system, and center of cancer nervous system that starts from the brain tissue and spinal cord. Other studies also show that the study of the environment and external condition, and also with the lifestyle of an individual will help in understanding more on the factors.

In Malaysia, there is a rise in the cancer disease affecting the community. It is listed as the top ten of death causing diseases in the country (Ministry of Health Malaysia, 2013). The most common cancer in the community is breast cancer, head and neck cancer, colorectal cancer, cancer of the trachea, bronchus and lungs, whilst lowest recorded of cancer is the cervices uteri. Breast cancer cases occur commonly in most countries such as in United Kingdom where it is predicted that every one of eight women will be diagnosed with breast cancer. In 2012, more than 464 000 cases of breast cancer, which is the sixth highest incident that occurs in Europe. Worldwide, there are over 1.68 million women are diagnosed with breast cancer and in the next forty years, it was estimated that their lifespan will increase (Siegel & Jiemin, 2014).

Breast cancer is the most common type of cancer. There are several causes for the breast cancer which includes the mutation of *BRCA1* and *BRCA2* through genetics, obesity, hormone replacement therapy, pills, and alcohol and through the lifestyle of the community (Parkin *et al.*, 2001). For women with *BRCA1* and *BRCA2* mutation have a higher risk of lifetime breast cancer and ovary cancer. They also have higher possibilities of having a risk in having pancreas cancer, and others. Aging increases the risk of breast cancer. At 70 years old, over 80% of women with *BRCA1* and *BRCA2* mutation are diagnosed of breast cancer and 60% of them have ovary cancer (Milne & Antoniou, 2011). Other than gene mutation, there are many more cancer forming factors. The external factors that promote cancer are the tobacco contents in the cigarette, alcohol intakes and the types of diet of red meat intake, obesity and environment (Greenwald, 2005). According to Rastogi (2009), the study of health and genomic are hard because the interaction of cell and protein that occur in the cell or body are complicated. It was estimated that even until 2020 the study of treatment for cancer is still limited (Anand *et al.*, 2008).

New approach as have been used in combating cancer development such as epigenetic of cancer, cancer cell stem, cancer immunology, and cancer metabolism. Cancer epigenetic study focuses on the deeper understanding of the ability of cell to

differentiate between regenerative tissue and control of cell division. The study of cancer immunology shows the ability of immune system to eliminate a cancerous cell, for example the application of dendritic cell in cancer immunotherapy. For cancer metabolism, a deeper research focuses on the ability of cancer cell to rejuvenate its energy for cell metabolism, which is still newly introduced (Yap, 2013). Other than these approach as in studying cancer, organization such as World Health Organization (WHO) (2014) also took a step in educating the community in the risk factors of cancer (World Health Organization, 2014).

Natural compounds in medicinal plants are being the major source for its ability to be applied in medicinal treatments. Drugs development using natural compound is increasing drastically for its high demands in modern medicine to control or combat diseases for example thyroid fever, gonorrhea, and tuberculosis. The increase in the human population also gives approach in search of many more medicinal sources that is effective and can be produced in shorter time. As for that, the study of natural compound in plants content is very important towards drug development for the global demands (Doughari, 2012). It is proven by many research that the natural compound of plants have many beneficial medicinal traits. An examples of natural compound that is used in medicinal purpose are *digoxin* and digitalis sp., quinine and quidine from *Cinchona* sp., vincristine and vinblastine from *Catharanthus roseus*, atropine from *Artopa* belladonna, and morphine and codeinefrom *Papaver somniferum*. At least 60% of antitumor drugs and anti-infective in drugs are from plants natural compound (Shu, 1997). Currently, a focus of studies on cancer cell is towards the activation of apoptotic process in cancer cell.

Developments of drug for medicinal purpose, treatment and therapy, natural compound was found to have the most effective in handling diseases. The use of natural compound with therapeutic traits has been applied since the early civilization. Before, the main sources of natural compound can be found in minerals, plants and animal products (De Pasquele, 1984). In the industrial revolution era and the growth of organic

chemical have triggered a more depth research in the ability of pharmaceutical treatments using synthetic product. Natural compound was found to be easier to obtain, safer and cheaper compared to synthetic drugs. Overall, 25% of medicine in the world are from plants and 121 of the active compounds consisted in this drugs (Rates, 2000). Based on World Health Organization (WHO), 11% of the 252 kinds of drugs and some synthetic drugs, are obtained from precursor plants. Extraction technique of natural compound is important to maximize the recovery of bioactive compounds. Traditional extraction involves Soxhlet extraction, solid-liquid extraction, and liquid-liquid extraction. The traditional techniques are still being applied today as it is more effective and cheaper in early stage (Ibanez *et al.*, 2012). A studies by Ibanez *et al.* (2012) have also introduced the extraction technique that can be applied in the future for a more effective production in the purpose of drug development in labs or industrial. A deeper knowledge in the extraction technique shows the importance of the plants extract ability in natural compound for drug development.

Combination of drugs are a novel step for future medicinal treatments. The reason is to enhance the effect of the drugs in treatments. Study shows that by combining the drugs give maximum changes in a treatment. Some may show an increase of its effects. In traditional methods, a combination of herbs and medicinal plants have long been practiced. By combining several kinds of extract or mixture of plants, it enhances the effects of the treatments from the plants itself (Adwan & Mhanna, 2008). Some studies have proven the combination effect of drugs in increasing the treatment efficiency. In addition, with the ability of combining different drugs, a different medicinal effect can occur also, in the interaction of the drugs content. The main focus of these studies in combining drugs are to test for synergistic effect in treatment. The synergistic effect can be practiced more in the studies as it can produce a more stable outcome and with less side effects. Synergy is defined as the interaction of an agent or energy that can combine or add or lessen its effect compared to individual use (Breitinger, 2012). The relationship of an active biological agent has the most important pharmacology and biomedicine effect. In synergy context, the effect of

the relationship in biological activity have been observed in some drugs. Certain situation that relates to these are:

- Combination of cytotoxic drugs in cancer treatment and infection needs low dose of drug in order for a therapeutic effect to work, with less side effects.
- The combination of antibiotic also gives a less side effect that gives little or lessen the effect of resistance.
- The effect of the drugs also adds other use of drugs and does not give its own effect alone.

These cases shows that the use of many mixtures of drugs does not only give synergistic effect, but also antagonistic or no effect at all. Current studies focus on the application of combined drugs that produce synergistic effect (Breitinger, 2012). Many research have applied synergistic effect and its ability to be applied in medicinal plants. Many types of natural compounds in medicinal plants always been combined in studies of medicinal plants. However, lots of these studies are currently in their preliminary stage. A deeper studies will open many doors in the future for understanding and development in drug development using plant extract. More studies on the drug interaction also help in understanding the application of the drugs in treatments. Therefore, this study was to investigate the antiproliferative effect of Acanthaceae plant extracts and also their combination effect on breast cancer cell line, MCF7.

1.2 Objectives

1. To examine the antiproliferative effect of Acanthaceae species plants using methanol and ethyl acetate from leaf and stem extracts of *Strobilanthes crispus* and *Ruellia tuberosa*, and methanol and ethyl acetate from root extract of *Clinacanthus nutans* towards breast cancer cell line, MCF7.
2. To study synergism in combining compound extract from *Strobilanthes crispus* and *Ruellia tuberosa* towards breast cancer cell line, MCF7.

CHAPTER 2

LITERATURE REVIEW

2.1 Cancer

Cancer is a disease in which abnormal cells divide without control and spread to other tissues. There are various types of cancer due to genetic changes in some period of time. Cancer can arise in various parts of the body's tissues with its own characteristics. Basis of the formation of most cancer have similarities in its process. The characteristics of a cancer cell is having an uncontrolled proliferation, lack in cell response signal, the ability of eternity, and the ability to a non-stop proliferation (Solomon *et al.,* 1999). Two main causes of the illness include a lack of biological nutrients received by the mitochondria, or failure of the nucleus in handling its purpose as the cell metabolic control center. Incidence of cancer cell are due to failure of the nucleus in controlling cell metabolism. Errors occurred give rises to the uncontrolled or multiplication of cells proliferation at the same time. This failure will allow the cell to exit the tissue and spread to other tissues, therefore, infecting non-infected tissues (Rath, 2001). There are two mechanisms of spread by the cancer, which are of infringing and metastasis. Infringing refers to migration and tissue penetration directly or to the nearby cell. Metastasis refers to the ability of cancer cells to enter into the lymph and blood vessels then spread to other tissues in the body (National Institutes of Health, 2012). This mechanism is the basic cause of the spread and formation of cancer cells.

Mutation in cellular function can cause cancer; either through somatic or inherited, through a single nucleotide protein (SNP), or a large genetic changes. The development of cancer is caused by various types of cellular level mutations. Other genetic mutation also causes defects on protein encoding centre and changes the normal cells genome to cancerous (Joseph *et al.*, 2012). Two characteristics of cancer is the uncontrolled multiplication of cells and extensive production of collagen dissolving enzyme. The spread of cancer cells uses the same concept with the spreading of normal cells but in uncontrollable condition. Mechanism that dissolves tissue helps the spreading of the cancer. Tissue that is exposed to cancer can be destroyed or suffer permanent damage (Rath, 2001).

2.1.1 Cancer Genetic

Cancer genetic only involved in one small unit in the human genes. The diversity of forms of cancer is determined by changes in gene function. It can be classified into three groups. The first group is a proto-promoting oncogenes that are capable of producing protein products that increases the doubling of cells or prevents normal cell death. This gene mutation is called oncogenes. The second group is tumor suppressors which produce proteins that prevent cell division or can cause cell death. Cells that carry mutated genes have failure in cell growth control. The third group is the genes containing DNA repair genes. This gene can stop mutation to cause cancer (Schneider, 2001).

Mutations in the DNA often occur due to environmental conditions. According to the study on the interaction of the tobacco, the tobacco effects need a long time until the cancer to formed (Dzivenu, 2003). However, one type of mutation is not enough to be form cancer cells. Multiple mutations are required to convert the normal cells to cancer cells.

2.1.2 Oncogenes

Oncogenes play an important role in the process of the formation of cancer cells. The doubling of normal cell is promoted by signals from a proto-oncogenes to proteins in the nucleus. A cascade of signal transduction occurs in the signal transfer. For a cascade of signal transduction in membrane receptors to occur a signal molecules is needed. Protein intermediates carry signals through the cytoplasm, and transcription factors in the nucleus to activate genes for cell division in a cascade of signal. Each protein kinase will be activated on an ongoing basis until it reaches the end of the transfer of the signal. Some proteins can activate more than one protein in the cell. Path of transduction shows the activation of cascade of signal in cell, just after cell receptors are activated, and eventually activating the transcription factor in cell (Schneider, 2001).

Oncogenes, a mutated proto-oncogenes, is a code for molecular signals which activates the cascade of signal which later will increase the production factors that stimulate cell growth. Alteration of the proto-oncogenes promotes oncogene arising from mutations and unfortunately contributes to cancer. Oncogenes are dominant mutations that actively controls expression of cell growth. However, it has been proven that oncogenes are not inherited in causing cancer (Schneider, 2001).

2.2 Cell cycle

Cell cycle is the process where cells go through cell growth and proliferation. In normal cells, the cell cycle is controlled by a series of complex routes signals where the cells grow, DNA replicates and doubled. This process involves a number of mechanisms to ensure that every problem is corrected, or for cell to go through apoptosis process. For cancer, genetic mutations affect the failure in the initial process which resulting in uncontrolled proliferation (Cyclacel Pharmaceuticals, 2014).

Division of normal cells are in orderly process and controlled in the cell cycle. The order of cell cycle can be seen in Figure 2.1. In accordance with the order; Gap 1 phase (G1 phase), the synthesis phase (phase S), Gap 2 phase (G2 phase), which is in between phase and mitosis phase. In phase G1 and G2, cell active in metabolism and divide. In S phase, copy of chromosome formed through DNA replication. In the phases of mitosis, the separation of nucleus and cytoplasm occurred (Schneider, 2001). This arrangement allows the process of cell cycle phase goes smooth and mitosis to continue. Thus, resulting in a new normal cells. However, mutations in the proto-oncogenes resulting in garbled or uncontrolled cell cycle resulting in the formation of cancer cells.

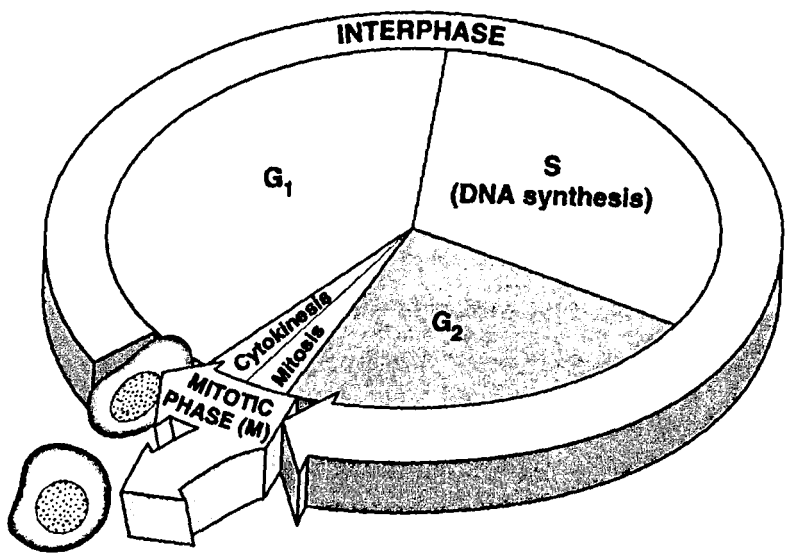

Figure 2.1: Cell cycle (Cummings, 2014).

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