ANTI-PROLIFERATIVE ACTIVITY OF *STROBILANTHES CRISPUS* ON BREAST CANCER CELL LINES

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DECLARATION

I hereby declare that the material in this thesis is my own except for quotations, equations, summaries and references, which have been duly acknowledged.

28 September 2018

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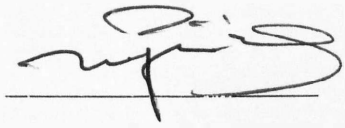

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ABSTRACT

In the advancement of chemical biology and combinatorial chemistry today, natural products remain a potent source of the anticancer drug development. *Strobilanthes crispus* (*S. crispus*) has been traditionally used as medicine in several countries and reported to have anticancer, antioxidant, free radical scavenging and antidiabetic activities. Thus, the aim of this study is to investigate the anti-proliferative effect of *S. crispus* on breast cancer cell lines and also its mode of action. The chemical compounds were extracted from leaves and stems of the plant through two types of extraction methods. First is single solvent extraction using methanol then followed by stepwise extraction using solvents of increasing polarity; hexane, chloroform, ethyl acetate and water. The *S. crispus* anti-proliferative activity on breast cancer cells was evaluated using MTT assay. Among the extracts, only five showed inhibition of cell proliferation in MCF-7. The best antiproliferative activity was observed in leaf water (LW) and stem ethyl acetate extracts (SE) with the IC_{50} value of 23 µg/ml and 38 µg/ml, respectively. However, the IC_{50} values for stem chloroform, leaf methanol and leaf chloroform extracts were at the range of 70-90 µg/ml. Meanwhile, only two extracts showed inhibition of cell proliferation in MDA-MB-231. They were leaf water and stem hexane extracts (SH) with the IC_{50} values of 40 µg/ml and 60 µg/ml respectively. Treatment with *S. crispus* extracts also caused morphological changes on both cell lines. Chromatin condensation and peripheral aggregation of nuclear chromatin were observed in the treated cells. The leaf water and stem ethyl acetate extracts were found to potentially have the apoptosis-inducing activity and cause cell accumulation in sub-G1 phase in MCF-7. The leaf water extract increased the expression of *PTEN* in MCF-7 and reduced the expression of *BCL-2* and *MCL-1* in MDA-MB-231. The stem hexane extract was also found to have potential in inducing apoptosis in MDA-MB-231 by decreasing both *BCL-2* and *BCL-XL* genes expression. The stem hexane extract was also found to interfere the cell cycle progression in MDA-MB-231 by supressing Cyclin A2 protein. In conclusion, the *S. crispus* has the potential to be developed into chemopreventive or chemotherapeutic agent.

ABSTRAK

AKTIVITI ANTI-PROLIFERATIF *Strobilanthes crispus* KEPADA TITISAN SEL KANSER PAYUDARA

Dalam kemajuan biologi kimia dan kimia gabungan, hasilan semulajadi kekal menjadi sumber utama kepada pembangunan ubat antikanser pada masa kini. Strobilanthes crispus (S. crispus) telah digunakan sebagai ubat tradisional di beberapa negara dan dilaporkan mempunyai komponen antikanser, antioksidan, pemusnah radikal bebas dan antidiabetic. Oleh itu, matlamat kajian ini adalah untuk mengkaji potensi S. crispus sebagai agen anti-proliferatif dan cara tindakannya. Sebatian kimia telah diekstrak dari tumbuhan melalui dua kaedah pengekstrakan; pengekstrakan menggunakan metanol kemudian diikuti dengan pengekstrakan berperingkat menggunakan pelarut heksana, kloroform, etil asetat dan air. Aktiviti anti-proliferatif S. crispus pada sel kanser payudara ditentukan melalui ujian MTT. Hanya lima ekstrak menunjukkan perencatan pertumbuhan sel dalam MCF-7. Aktiviti antiproliferatif yang terbaik diperhatikan dalam ekstrak air daun (LW) dan etil asetat batang (SE) dengan nilai IC_{50} masing-masing 23 µg/ml dan 38 µg/ml. Manakala, nilai IC_{50} untuk ekstrak kloroform batang, metanol daun dan kloroform daun adalah pada lingkungan 70-90 µg/ml. Sementara itu, hanya dua ekstrak menyebabkan perencatan proliferasi sel dalam MDA-MB-231 iaitu ekstrak air daun dan heksana batang (SH) dengan nilai IC_{50} masing-masing 40 µg/ml and 60 µg/ml. Rawatan dengan ekstrak S. crispus menyebabkan perubahan morfologi pada kedua-dua titisan sel. Ekstrak air daun dan etil asetat batang didapati mempunyai potensi pengaruh apoptosis dan menyebabkan pengumpulan sel pada fasa sub-G1 dalam MCF-7. Ekstrak air daun meningkatkan ekspresi gen PTEN dalam MCF-7 dan mengurangkan ekspresi gen BCL-2 dan MCL-1 dalam MDA-MB-231. Ekstrak heksana batang mengaruh apoptosis dalam MDA-MB-231 dengan mengurangkan ekspresi gen BCL-2 dan BCL-XL. Ekstrak heksana batang juga mengganggu kitaran sel dengan mengawal ekpresi protein Cyclin A2 dalam sel MDA-MB-231. Sebagai kesimpulan, S. crispus mempunyai potensi untuk dikembangkan menjadi agen chemopreventif atau kemoterapi.

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LIST OF SYMBOLS

°C	-	Celsius
%	-	Percent
g	-	Gram
µg	-	Microgram
µl	-	Microliter
ml	-	Milliliter
µg/ml	-	Microgram per mililiter
ng	-	Nanogram
ng/ml	-	Nanogram per milliliter
rpm	-	Rotation per minute
bp	-	Base pair
kDa	-	Kilodalton

LIST OF ABBREVIATIONS

SD	-	Standard deviation
IC_{50}	-	50% inhibitory concentration
UV	-	Ultraviolet

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Cancers are major public health problem in many parts of the world. It is also one of the principal causes of mortality and morbidity among population around the world. In 2017, approximately 1688780 new cancer cases and 600920 cancer deaths are estimated in the United State alone (Iqbal, Abbasi, Mahmood, Kanwal, Ali and Khalil, 2017). In Malaysia, cancer is also reported to be one of the common causes of death. Among several types of cancer, breast cancer is reported as the most frequent cancer among women in Malaysia (Omar and Ibrahim Tamin, 2011).

There were many factors that can lead to the initiation and progression of cancer. It is reported that the development of cancer can be triggered by external and internal factors. The external factors include the smoking habit, improper diet, alcohol use, exposure to radiation and pollution, etc. (Sloan and Gelband, 2007). The development of cancer can also be induced by internal factors, such as hormonal and body immune system disorder, genetic instability, mutation in DNA repair genes, inactivation of tumor suppressor genes and activation of oncogenes (Parsa, 2012).

At present, many researches have been conducted on cancer treatment and several mechanisms have been discovered to control the proliferation of cancer cells such as by targeting certain gene or pathway. It includes the targeting of apoptosis pathway (Li-Weber, 2013). Apoptosis is a programmed cell death where the cells were forced to commit suicide. Induction of apoptosis in cancer cells can stop the proliferation and thus prevent the cancer cells from spreading (Ghobrial, Witzig and Adjei, 2005). Other studies also attempt to manage the proliferation of cancer cell through cell cycle alteration (Hadad, Hardie, Appleyard and Thompson, 2014). Cell cycle is where cells are undergoing division and result in cell growth. Therefore, the arrest in cell cycle can halt the cancer cells from propagate (Waldman, Zhang, Dillehay, Yu, Kinzler, Vogelstein and Williams, 1997).

There are several cancer treatments that have been available for cancer patients nowadays which include surgery, chemotherapy, radiation therapy and hormone therapy (Miller, Siegel, Lin, Mariotto, Kramer, Rowland, Stein, Alteri and Jemal, 2016). Although surgery remains the main choice for cancer treatment (Yip, Bhoo and Teo, 2014), some patients prefer to consume medicine for prolonged period of time as adjuvant therapy. However, most of the medicines are synthetic; very expensive (Kantarjian and Rajkumar, 2015) and reported to give many side effects (Florea and Büsselberg, 2011).

Natural cancer treatment by using natural products is recommended by some physicians. Natural products have been used as a chemopreventive agent in preventing the initiation, promotion, and progression of cancer (Thangapazham, Sharma and Maheshwari, 2006). There are abundance of natural bioactive compounds have been identified. The natural bioactive compounds were reported to be useful in cancer prevention and therapy by targeting various signaling molecules and pathways (Bishayee and Sethi, 2016). Natural cancer treatment is advantageous as the natural product compounds have fewer side effects and lower toxicity compared to synthetic compounds (Ko and Moon, 2015).

Strobilanthes crispus (*S.crispus*) is also called as "daun picah beling", "enyoh kelo", "kecilbeling", "kejibeling", "ngokilo" in Jakarta and Java. This plant is also known as "pecah beling", "bayam karang", "pecah kaca", "jin batu" in Malaysia. The plant can attain a maximum height of 0.5-1.0 meters. They can be found on riverbanks or abandoned fields. The *S. crispus* has been used as a traditional medicine for variety of ailments and reported to have multiple health benefit effect (Nurraihana and Norfarizan, 2013). The *S. crispus* also has been reported to have anticancer effects against several types of cancer cell lines (Muslim, Ng, Itam, Nassar, Ismail and Abdul Majid, 2010).

Despite being traditionally used by indigenous people in Sabah so far no study was done on the Sabah-originated *S. crispus*. Thus, this study was intended to examine the anti-cancer property of *S. crispus* in breast cancer that originated from Sabah. This research also makes use of both leaf and stem parts of the plant while the other research mainly focusing only in the leaf part. Different approach also was done in the plant extraction method in order to maximize the extraction of bioactive compounds. Thus, this study was aimed to investigate the potential of antiproliferative activity of *S. crispus* and also to understanding its underlying mechanisms towards breast cancer cells.

1.2 Research Hypothesis

The *S. crispus* extracts can inhibit the proliferation of breast cancer cells and may induce apoptosis or cell cycle arrest to the cells.

1.3 Research Objectives

Different from other researches, the *S. crispus* was extracted using two types of extraction method in this study. The single solvent extraction and followed by liquid-liquid partition by which might enhance the pharmacological properties the *S. crispus* extracts. Thus, the objectives of this study were to examine anti-proliferative activity of the *S. crispus* extracts and their effects on apoptosis and cell cycle of breast cancer cell lines.

CHAPTER 2

LITERATURE REVIEW

2.1 Breast Cancer

In the United State, a total of 1,665,540 new cancer cases were reported in 2014 (Siegel, Ma, Zou and Jemal, 2014) and researchers found that one of 8 women will develop breast cancer (DeSantis, Ma, Bryan and Jemal, 2014). In Peninsular Malaysia, 21,773 cancer cases were reported to National Cancer Registry in 2006 (Omar and Ibrahim Tamin, 2011). Then, the number of cases was continuously increased from 32,000 new cases in 2008 to about 37,000 in 2012. In the period from 2007 to 2011, about 64,275 medically certified and non-medically certified cancer deaths were reported by the National Registration Department in Malaysia (Azizah, Norsaleha, Noor Hashimah, Asmah and Mastulu, 2016).

The ten most frequent cancers in Malaysia are the breast, colorectal, lung, nasopharynx, cervix uteri, lymphoma, leukaemia, ovary stomach and liver cancers. Breast cancer was reported to be the most common cancer among women in Malaysia. In 2006, breast cancer cases were 29.9% of the total female cancer cases registered in National Cancer Registry. Then, the number was increase to 32.1% in 2007 (figure 2.1). Among the breast cancer cases, most of the cases were from 50-59 age groups and reported to be more predominant among Chinese

as compared to Malay and India. In 2017, breast cancer is estimated to have 252,710 new cases and 40,610 deaths in world's population (Iqbal *et al.,* 2017).

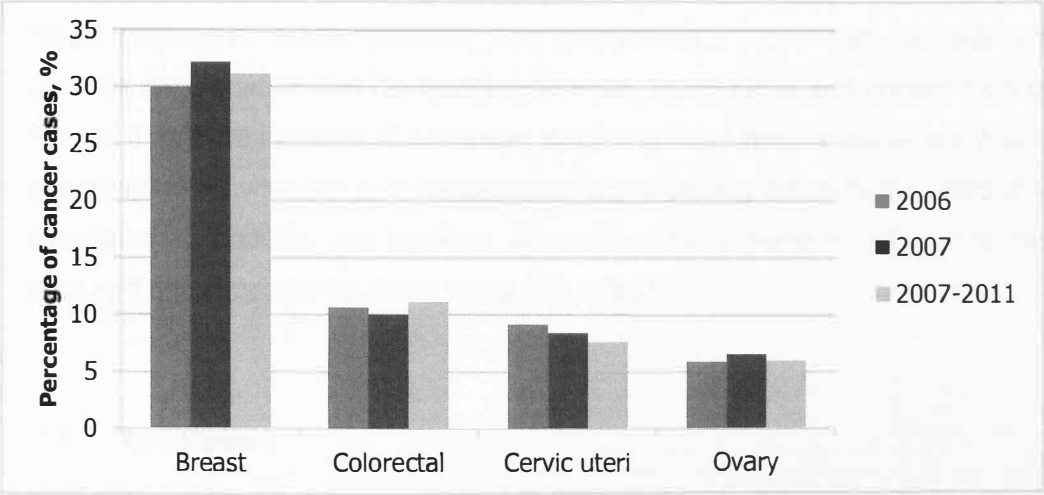

Figure 2.1 : The cancer statistic in 2006 to 2011. The figure showed the most common cancer among females in peninsular Malaysia in 2006 to 2011.

Source : Malaysia Cancer Statistics-data and figure Peninsular Malaysia 2006 and 2007; Malaysian National Cancer Registry Report 2007–2011.

There are many factors that can lead to breast cancer development. Among the well-known risk factors are nulliparity family history, not breast feeding and use of oral contraceptives (Yip *et al.*, 2014). Physical inactivity, alcohol use, overweight and obesity were also known to be the risk factors for breast cancer (Sloan and Gelband, 2007). Genetic predisposition is one of the most dominant risk factor of breast cancer as approximately 15% of breast cancer patients have family history of breast and ovary cancers. Nevertheless, cancer also can be caused by genetic mutation in DNA repair genes (*p21, p22, p27, p52* and *p53*), tumor suppressor genes (*p53, NF1, NF2* and *RB*), oncogenes (*MYC, RAF, BCL-2* and *RAS*) and genes involved in cell growth metabolism (Iqbal *et al.,* 2017).

Several types of treatment are available for cancer patients nowadays including surgery, chemotherapy, radiation and hormone therapy together with drug or medicine consumption. Chemotherapy is reported to help improving survival of many breast cancer patients. However, the side effects have been the major concern to cancer patients. The chemotherapy can affect the patient's immune system which can cause hypercalcemia, neutropenia and preservation of fertility. There are numbers of anticancer drugs that have been available and highly effective against wide range of cancers such as irinotecan, doxorubicin, oxaliplatin, melphalan, carboplatin, etc. However these drugs are expensive, not ecofriendly, toxic and most importantly giving many side effects.

2.2 Apoptosis

Cancer can be a product of some events occurred in the cell. One of it may due to the deregulated cell proliferation together with suppression of apoptosis that needed to encounter it (Evan and Vousden, 2001). Apoptosis plays an important role in development and progression of cancer. According to Kasibhatla and Tseng (2003), defective apoptosis is a major factor in the development and progression of cancer. While, induction of apoptosis helps the elimination of cancer cells by causing rapid death to the cells (Thompson, 1995). It is well documented that most of cytotoxic anticancer agents induce apoptosis (Lowe and Lin, 2000).

Apoptosis is sometimes referred as programmed cell death where the cells were forced to commit suicide. Caspases and DNases enzymes take parts in participate directly or indirectly in nuclear condensation of chromatin and inducing the death of the cell (Zamzami and Kroemer, 1999). Apoptosis regulated by the expression of Bcl-2 and Bax proteins which are a product of genes regulation apoptosis (Lichnovský, Prochazkova, Erdösová, Nepozitkova, and Kolar, 1999).