EFFECT OF RED ONION (Allium cepa) PEEL EXTRACTS AND FRACTIONS ON CELL PROLIFERATIVE AND METASTATIC ACTIVITIES IN MDA-MB-231

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by

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LIST OF ABBREVIATIONS

APC	Adenomatous polyposis coli
APS	Ammonium persulfate
AKT	Protein kinase B
BSA	Bovine serum albumin
Cal-27	Human tongue squamous cell carcinoma cell (ATCC® CRL- 2095™)
CDK	Cyclin dependent-kinase
CK1	Casein kinase 1
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DVL	Dishevelled
E1	Red onion peel ethanol extract
E3	Red onion peel water extract
ECL	Enhanced chemiluminescence
ECM	Extracellular matrix
EDTA	Ethylenediaminetetraacetic acid
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
EtBr	Ethidium bromide
F1	Red onion peel ethanol Fraction 1
F1W	Red onion peel water Fraction 1
F2	Red onion peel ethanol Fraction 2
F2W	Red onion peel water Fraction 2

FACS	Fluorescence-activated cell sorting
FBS	Fetal bovine serum
FZD	Frizzled
GSK-3	Glycogen synthase kinase-3
KDa	Kilo Dalton
LEF-1	Lymphoid enhancer factor-1
LRP	Low-density-lipoprotein-receptor-related protein
MAb	Monoclonal antibody
MAPK	Mitogen-activated protein kinase
MDA-MB-231	Mammalian gland/breast derived from metastatic site
MCF-7	Mammalian breast cancer cell
MCF-10a	Non cancer origin breast cell line
MMP	Matrix metalloproteinase
M-Per	Mammalian protein extraction reagent
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium bromide)
OSCC	Oral squamous cell carcinomas
PAGE	Polyarylamide gel electrophoresis
PBS	Phosphate buffered saline
PCP	Planar cell polarity
PI	Propidium iodide
PI3k	Phosphatidylinositol 3-kinase
PVDF	Polyvinyl difluoride

SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SEM	Standard error of the mean
TBE	Tris-borate-ethylenediaminetetraacetic acid
TBS	Tris buffered saline
TBST	Tris buffered saline- Tween® 20 buffer
TEMED	N,N,N',N'- teteramethylethylenediamine
USM	Universiti Sains Malaysia
UV	Ultraviolet
Wnt	Wingless and integration site growth factor

KESAN EKSTRAK DAN PECAHAN KULIT BAWANG MERAH (*Allium cepa*) PADA AKTIVITI-AKTIVITI PROLIFERATIF DAN METASTATIK SEL DI MDA-MB-231

ABSTRAK

Kanser merupakan punca utama kematian di Malaysia dan juga negara-negara yang sedang membangun. Penyakit tersebut menyebabkan sel-sel tidak normal membahagi tanpa kawalan serta berupaya menyerang tisu normal. Walaupun terdapat perkembangan dalam rawatan kanser, kadar kematian kanser masih tinggi terutamanya pesakit kanser yang invasif. Oleh itu, penemuan anti-kanser ejen yang berkesan daripada semua sumber perlu dilakukan. Flavonoid, seperti quercetin, boleh didapati dalam kandungan yang tinggi dalam isi bawang dan juga kulit bawang. Flavonoid telah dibuktikan dapat menggalak aktiviti anti-proliferatif dan berpotensi anti-kanser. Oleh sebab itu, kajian ini dijalankan dengan objektif untuk mengkaji anti-kanser aktiviti ekstrak dan pecahan kulit bawang merah (*Allium cepa*) terhadap MDA-MB-231, MCF-7 dan Cal-27. Kesan ekstrak dan pecahan kulit bawang merah dalam aktiviti anti-proliferatif, induksi apoptosis-kematian sel diprogram dan anti-metastasis telah dipelajari. Di samping itu, penglibatan laluan isyarat Wnt dalam aktiviti anti-kanser ekstrak dan pecahan kulit bawang merah juga disiasat. Ekstrak (E1 dan E3) dan pecahan (F1, F2, F1W dan F2W) menunjukkan kesan melarang daya maju sel MDA-MB-231. Walau bagaimanapun, nilai IC_{50} ekstrak dan pecahan kulit bawang merah tidak mendorong apoptosis dalam MDA-MB-231. Dalam analisis kitaran sel, hanya E1 dan F1 menjejaskan kitar sel MDA-MB-231 selepas rawatan selama 24 jam, 48 jam dan 72 jam. Di samping itu, E1, F1, F2, E3, F1W dan F2W menunjuk aktiviti anti-metastatik melalui perencatan aktiviti lekatan sel-

fibronektin, penghijrahan sel dan pencerobohan sel. Analisis Western blotting menunjukkan bahawa aktiviti anti-kanser ekstrak dan pecahan kulit bawang merah tidak dikaitkan dengan laluan isyarat Wnt. E1 dan F1 cenderung untuk mempengaruhi tahap ekspresi Akt fosforilasi (Ser473) dalam MDA-MB-231. Selain itu, kajian tersebut juga mengesan perubahan tahap ekspresi β-catenin fosforilasi (Ser33) dalam MDA-MB-231 yang dirawat dengan E1 selama 24 jam. Oleh itu, laluan yang mungkin dicetuskan oleh E1 dalam MDA-MB-231 adalah melalui laluan isyarat bergantung Akt dan β-catenin, manakala F1 mungkin menunjuk anti-kanser aktiviti melalui Akt sahaja, seperti yang ditunjukkan dalam kajian tersebut. Tahap ekspresi protein β-catenin fosforilasi (Ser552) menurun dalam MDA-MB-231 yang dirawat dengan E1, E3, F1W dan F2W. Ini menunjukkan bahawa E3, F1W dan F2W juga mendorong aktiviti proliferatif dan anti-metastatik melalui laluan β-catenin. Sebaliknya, F2 mungkin menunjukkan aktiviti anti-kanser melalui laluan yang berlainan daripada laluan isyarat Wnt. Kajian ini memberi maklumat berguna tentang kesan anti-kanser dari ekstrak dan pecahan kulit bawang merah dalam kanser payudara, di mana ekstrak dan pecahan tersebut boleh menjadi ejen anti kanser yang berkesan untuk mengatasi rintangan terapi kanser dan untuk menghasilkan terapi novel untuk kanser.

EFFECT OF RED ONION (*Allium cepa*) PEEL EXTRACTS AND FRACTIONS ON CELL PROLIFERATIVE AND METASTATIC ACTIVITIES IN MDA-MB-231

ABSTRACT

Cancer is the leading cause of mortality in Malaysia and other developing countries. The disease causes the abnormal cells divide uncontrollably and have the ability to invade normal tissues. Although there are advanced cancer treatments being developed, the mortality rate due to cancer remains high especially in the patients with invasive cancers. As such, there is a need to discover more effective anti-cancer agents from various resources. Flavonoids, such as quercetin, are found in high levels in onions bulb and onion peel. Flavonoids have been demonstrated to induce anti-proliferative and potential anti-cancer activities. For the reason, the present study was conducted with the objective to study the anti-cancer property of red onion (*Allium cepa*) peel extracts and its fractions by investigating the effects in MDA-MB-231, MCF-7 and Cal-27. The effects of red onion peel extracts and its fractions in the anti-proliferative activity, induction of apoptosis - programmed cell death and anti-metastasis were studied. In addition, the involvement of Wnt signalling pathway in the anti-cancer activity of red onion peel extracts and its fractions was also investigated. In the results, the extracts (E1 and E3) and fractions (F1, F2, F1W and F2W) showed promising inhibitory effect on cell viability of MDA-MB-231. However, the IC_{50} values of the extracts and fractions did not induce apoptosis in MDA-MB-231. In cell cycle analysis, only E1 and F1 affected the cell cycle of MDA-MB-231 after 24 hours, 48 hours and 72 hours treatments. In addition, E1, F1, F2, E3, F1W and F2W exerted anti-metastatic activity *via* inhibition of cell-

fibronectin adhesion activity, cell migration and cell invasion. Western blotting analysis demonstrated that the anti-cancer activity of red onion peel extracts and its fractions was not associated with Wnt signalling pathway. E1 and F1 tended to affect the expression level of phosphorylated Akt (Ser473) in the treated cells. Moreover, the study also detected changes in the expression level of phosphorylated β-catenin (Ser33) in MDA-MB-231 treated with E1 for 24 hours. Therefore, the possible pathways triggered by E1 in MDA-MB-231 were likely *via* Akt and β-catenin dependent pathways, whereas F1 might exert the anti-cancer activity *via* inhibition of Akt only, as determined in this study. The protein expression level of phosphorylated β-catenin (Ser552) was down-regulated in E1, E3, F1W and F2W treated MDA-MB-231, indicating that E3, F1W and F2W might also exert anti-cell proliferative and anti-metastatic activities *via* inactivation of β-catenin pathway. On the other hand, F2 might exhibit the anti-cancer activity *via* different pathways, which is independent of Wnt signaling pathway. The study provides useful information on the anti-cancer effect of the red onion peel extracts and its fractions in breast cancer, where the test extracts and fractions may be effective anti-cancer agents for overcoming cancer therapy resistance and for designing novel therapy for human cancers.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Globally, over six million people die because of cancers. It is estimated that thirty million of new cancer cases will occur by 2030 (Katikireddi & Setty, 2013). Cancer cells are different than normal cells as abnormal cells metastasize and grow uncontrollably (Zhou, 2014). Cancerous cells often invade neighbouring tissues and develop secondary tumors at distant sites (Alabsi *et al*., 2012). Cancerous cells are proven to express the traits of sustainable proliferation, cell death resistance, angiogenesis, immortality, invasion and metastasis (Hanahan & Weinberg, 2011).

Breast cancer-related mortality is increasing in developing countries in relation to the genetic factor, cultural diversity, ineffective treatments and late stage diagnosis (Bines & Eniu, 2008). The currently available treatments for this cancer are surgical, radiation and chemotherapy. On the other hand, oral cancer is the sixth most common cancer in the world, which accounts for up to 40% of cancer cases diagnosed in India and South East Asia. However, this cancer is not common in western countries.

Natural products, such as garlic, shallot and onion, have been proven to have *in vitro* anti-cancer property *via* inhibiting the growth of cancer cells or induction of apoptosis (Azadi *et al*., 2008; Griffiths *et al*., 2002). The products usually contain very high natural flavonoid. Onions (*Allium cepa* L.) belongs to *Allium* plants that can be found worldwide (Griffiths *et al*., 2002). Flavonoids and alk(en)yl cysteine

sulphoxides (ACSOs) in onions are found to be beneficial in human health (Griffiths *et al*., 2002).

The study aimed to determine the cytotoxic effect of red onion (*Allium cepa*) peels in MCF-7, MDA-MB-231 and Cal-27. Moreover, the anti-metastasis activity and the association of aforementioned cellular processes with Wnt signaling pathway triggered by the red onion peel extracts and its fractions on MDA-MB-231 were investigated.

1.2 Problem statements

Cancers have claimed over millions of lives every year. It is estimated that approximately 1,688,780 new cancer cases happen in 2017 (American Cancer Society, 2017). The World Health Organization (WHO) estimated that the number of cancer deaths will increase to 13.1 million by 2030 (Farooqui *et al*., 2013). Around 6,000,920 Americans are expected to die because of cancers (American Cancer Society, 2017). It is estimated that about 252,710 women and 2,470 men in the US will be diagnosed with breast cancer in 2017.

Currently, there is no effective treatment for human cancers with least side effects even though many studies have been conducted in the search for potential anti-cancer agents. Current cancer treatments, such as surgery, chemotherapy and radiotherapy, have a wide range of side effects and deficiencies (Shahneh *et al*., 2013). Current cancer treatments possess adverse side effects, such as pain, vomiting, and weakened the immune system. Furthermore, cancer cells could develop resistance towards the chemotherapy. Moreover, aggressive cancers, such as triple

negative breast cancers (TNBC), have a poor prognosis and low survival rate. The long term recurrent rate of breast cancer remains high and metastatic relapse remains an incurable disease (Li *et al*., 2014). Cancer treatments have limited efficacy against TNBC. Moreover, the outcomes of current cancer treatments are unsatisfactory, particularly on the metastasis. Metastasis is the major cause of cancer deaths. Metastasis is the spread of cancer cells from the primary site to the distant location. It is a multi-step process. Cancer cells acquire cell motility, cell migration, cell invasion and angiogenesis during metastasis (Deep & Agarwal, 2010). Current cancer treatments have limited effect against metastatic cancer (Deep & Agarwal, 2010). Thus, it is very important to find new alternative cancer treatments for metastasis.

Onions are one of the major vegetables consumed in Malaysia, and hence, onion peels are the most common waste discarded in our kitchens (Abdullah *et al*., 2013). Onions are also consumed widely in other countries. More than 450,000 tonnes of onion waste is produced in the European Union every year (Roldan *et al*., 2008). It is reported that red onion contains a higher amount of total flavonoids than the white and yellow onions (Shon *et al*., 2004). In addition, the outer skin of red onions had higher antioxidant activity than yellow and white onions. Red onion contains organosulfur compounds and flavonoids, such as kaempferol, quercetin and anthocyanins. According to Griffiths *et al*. (2002), onions have anti-carcinogenic property, antibiotic activity, antioxidant property, lower blood cholesterol level, anti-cancer activity and property to reduce lipid peroxidation. Red onions have a higher yield of phenol and total flavonoid contents than white and yellow onions (Shon *et al.*, 2004). Red onion has been demonstrated to contain quercetin, anthocyanins and

polyphenolic compounds that display anti-cancer property. Therefore, the study was conducted to determine the anti-cancer activity of the red onion peel extracts and its fractions in human cancer cells.

1.3 Significance of the study

As the study focus on the cytotoxic activity, apoptosis-programmed cell death and anti-metastatic property in breast cancer cell lines, as well as the association of aforementioned cellular processes with Wnt signaling pathway, the outcomes of this study may provide a framework on the effectiveness of red onion peel extracts and fractions against TNBC. This study has an impact on drug discovery as it provides the translational potential for cancer treatment, particularly the metastatic property on breast cancer. Moreover, the waste of red onion could be utilized for anti-cancer approaches. In addition, the mechanism of the anti-cancer property of the red onion peel extracts and its fractions are illustrated. Furthermore, cost effective health supplements that complement cancer therapy could be introduced using the red onion peel extracts and fractions.

1.4 Objectives

The main objective of this study was to investigate the anti-cancer property of the red onion peel extracts and its fractions. The flow chart of the methodology is shown in Figure 1.

To do so, four specific objectives of the project are represented as follows:

1. To evaluate the cytotoxic effect of the red onion peel extracts and its fractions in human cancer cell lines using MTT assay.
2. To investigate the programmed cell death induced by the red onion peel extracts and its fractions in MDA-MB-231 using DNA fragmentation assay, Cell staining and Flow cytometry.
3. To determine the anti-metastatic effect of the red onion peel extracts and its fractions in MDA- MB-231 using Cell-fibronectin adhesion, Wound healing cell migration, Boyden chamber motility and Matrigel invasion assays.
4. To study the association of aforementioned cellular processes with Wnt signaling pathway using Western blotting.

Flow Chart

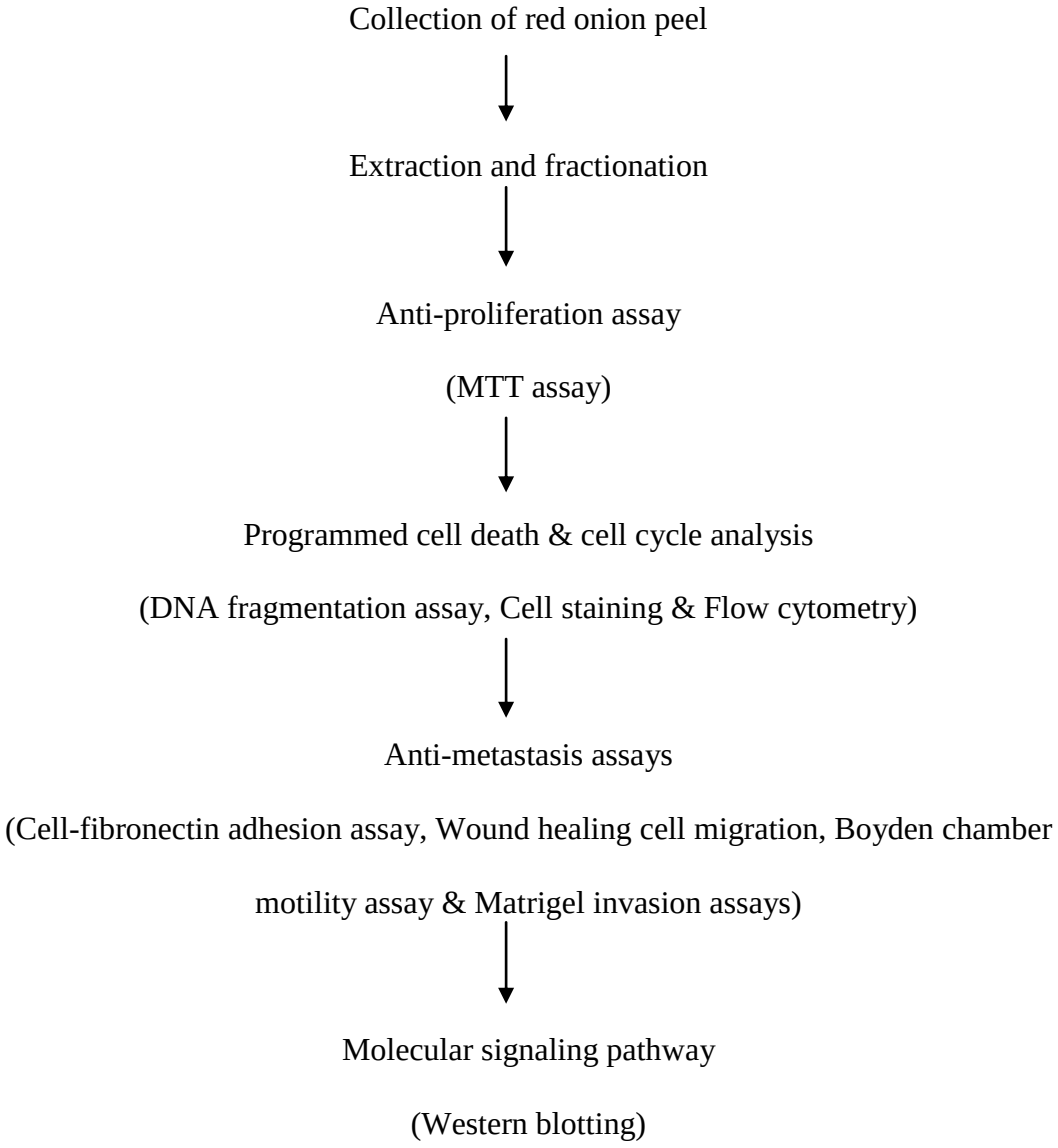

Figure 1: Flow chart of the methodology of this study.

CHAPTER 2

LITERATURE REVIEW

2.1 Oncogenesis

It is reported that one out of two men and one out of three women are diagnosed with cancers in their lifetime (Chial, 2008). This phenomenon is due to the close association of carcinogenesis that harboring mutations. Mutations are the hallmark of cancer (Loeb & Loeb, 2000). It is also one of the factors that contribute to the cancer formation. Mutations occur due to external factors, such as carcinogens, industrial chemicals and endogenous sources, including free radicals, DNA polymerase errors, depurination, chromosomal aberrations and others (Loeb & Loeb, 2000).

Cancer is a heterogeneous disease with deregulation of multiple cell signaling pathways. It is reported over hundreds of genes have been revealed to regulate tumour initiation and progression, where cellular transformation happens through the accumulation of cellular mutation and epigenetic changes (Pedraza-Farina, 2006; Jeggo *et al*., 2016). Mutations regulate the increased expression of oncogenes; the mutated form of normal cellular genes (proto-oncogenes) (Pedraza-Farina, 2006). Proto-oncogenes are a group of normal genes that could cause cancer formation when the genes are mutated. Oncogenes encode proteins that are usually involved in cell division, inhibition of cell differentiation and evasion of cell death (Chial, 2008). The underlying mechanism associated with mutation of proto-oncogenes are the point mutation, deletions or insertion, chromosomal translocation that lead to hyperactive gene products, increased transcription, higher gene and protein

expression (Chial, 2008). Activation of oncogenes stimulates the growth of cancer cells (Pedraza-Farina, 2006; Jeggo *et al*., 2016). Tumor suppressor genes, on the other hand, act as negative regulators of cellular growth. It is reported that mutation of a single oncogene or tumour suppressor gene is not sufficient to induce cancer formation (Pedraza-Farina, 2006). Oncogenesis could occur *via* single gene disorder, multiple genes disorder and chromosome disorder (Liu *et al*., 2011), where EGFR, PDGFR, Her2/neu, cyclin D, CDK4 and β-catenin are the examples of oncogenes, whereas TGF-βR, p53, bcl-2, bax, APC, PTEN are the selected examples of tumour suppressors (Malarkey *et al*., 2013).

2.2 Cancer

Cancer is one of the deadly diseases in Malaysia. In 2007, about 18,219 new cancer cases are registered in National Cancer Registry (Bain *et al*., 2014). Lung cancer, colorectal cancer, nasopharynx cancer, prostate cancer and lymphoma were the most common cancers found among Malaysian males, whereas breast cancer, colorectal cancer, cervix cancer, ovary cancer and lung cancer were the common cancers found in females (Zainal Ariffin & Nor Saleha, 2011). As a matter of fact, cancer is the major health concern now globally.

Several important alterations occur in order for the transformation of cancerous cells to happen. The alternations, including increased growth signals, resistant towards anti-growth signals, the escape of apoptosis, angiogenesis, unlimited potential in replicative, invasion, metastasis and genome instability (Hanahan & Weinberg, 2011). Normal cells require growth signal for entering proliferative phase. However, cancerous cells can generate growth factors to sustain

the growth; therefore, it reduces their dependence on mitogenic growth signals (Hanahan & Weinberg, 2011). Anti-growth signals can inhibit proliferative state of the cells temporarily or permanently.

Cancer is a disease, in which the cells grow uncontrolled and spread as abnormal cells. It has been demonstrated that this disease is caused by unhealthy diets, infectious organisms, tobacco, inherited genetic mutations and immune conditions (American Cancer Society, 2016). Cancer has higher mortality rate than acquired immune deficiency syndrome (AIDS), tuberculosis and malaria (American Cancer Society, 2016). Globally, one in seven deaths is associated with cancers. In 2012, there were 14.1 million new cancer cases diagnosed worldwide, whereby 8.2 million cancer deaths happened worldwide; 2.9 million in developed countries and 5.3 million in developing countries. It is expected that 21.7 million new cancer cases will be occurred by 2030.

The three most common cancers in economically developed countries are prostate cancer, lung cancer and colorectal cancer among men; and breast cancer, colorectal cancer and lung cancer among women (American Cancer Society, 2016). For economically developing countries, the three most common cancers are lung cancer, liver cancer and stomach cancer in male; and breast cancer, cervix uteri cancer and lung cancer in females. Different geographic areas cause different types of cancers. Breast cancer was the most common cancer diagnosed among women in 19 out of 21 countries (American Cancer Society, 2016). Age structure of the population, the availability and use of diagnostic tests, and the quality of treatments are the factors result in geographic differences in cancer occurrence.

2.3 Breast cancer

Breast cancer is the disease that formed in the tissues of the breast. Both men and women can be affected by this disease. In Malaysia, about 1 in 20 women are diagnosed with breast cancer (Musa *et al*., 2011). Breast cancer is the second most commonly diagnosed cancer in the world (Ferlay *et al.*, 2015). Breast cancer is at the highest rank of women's cancer that prone to the ratio of 1:20 in Malaysia (Yip *et al*., 2006). About 1 million new cases of breast cancer are registered annually worldwide (McPherson *et al*., 2000). Breast cancer patients have better survival rate if the disease is detected early (Nor-Afiah *et al.*, 2011). It was reported that breast cancer ranked as the fifth of the most cancer death with approximately 458,000 of deaths occur worldwide (Jemal *et al*, 2011).

In economically developed countries, the breast cancer patients tend to have good prognosis than the patients in developing countries (American Cancer Society, 2016). Asian countries have the largest numbers of breast cancer incidences, with 39% of the new cases diagnosed and 44% of cancer death. The United States and Canada have detected 15% of the new breast cancer cases and 9% of breast cancer deaths, whereas African countries account for 8% of the new breast cancer cases and 12% of breast cancer death (American Cancer Society, 2016).

It is reported that geographic differences affect breast cancer rate. Northern America, Australia, and Northern and Western Europe have higher incidence rates than Asia and Africa. The variation might due to different lifestyles and environmental factors. For example, 19.3 per 100,000 women in Eastern Africa and 89.9 per 100,000 women in Western Europe are diagnosed breast cancer (Curado,

2011). As for the Africa, South African Republic has the highest incidence rate of breast cancer, whereas Sudan and Ghana have the lowest incidence rates in Africa (Curado, 2011). In Asia, breast cancer incidence is lower than Western countries. Nevertheless, breast cancer remains a leading type of women's cancer in Asia. Singapore, Japan and Hong Kong have the highest rate of breast cancer incidence than India, Thailand and Korea (Curada, 2011). In Japan and Hong Kong, the most affected women are aged 45-50 years old, while this age group of Indian women is less affected. There is the difference in peak age for breast cancer patients in Asian countries and Western countries. The peak age of breast cancer patients in Asian countries is between 40-50 years old, and in Western countries, the peak age is between 60-70 years old (Curada, 2011).

The symptoms of breast cancer are skin dimpling, changes in skin colour or texture and the lump in the breast. The early stage of breast cancer has potential to be cured with 84% of five-year survival rate, while for the advanced stage of cancer, only 18% of five-year survival rate is predicted. Breast cancer usually develops from non-metastatic and hormone-dependent to highly invasive and hormone-independent properties.

Alcohol consumption is one of the factors that increase the occurrence of breast cancer among women. However, a study showed consumers of wine had a lower risk of getting breast cancer compared to none wine drinkers as the presence of polyphenols particularly anthocyanins and resveratrol in wine (Delmas *et al*., 2006; Arranz *et al*., 2012). Resveratrol inhibits tumor growth and induces apoptosis *in vitro* (Delmas *et al*., 2006). Breast cancer can be categorized based on the expression of

receptors, such as estrogen receptor (ER), Her2 receptor and progesterone receptor (Pervaiz *et al*., 2015). These markers could be the target for cancer treatment. Her2 belongs to type 1 receptor tyrosine kinase family and it was found that the expression of Her2 is upregulated by 25-30% in human breast cancer (Barros *et al*., 2010). However, no specific treatments are available for both ER- and Her2 negative tumors till now (Huang *et al*., 2013).

2.4 Breast cancer risk factors

Breast cancer is the major public health problem among females. The risk factors, such as the late age of first pregnancy, lower age of menarche, obesity, alcohol consumption, inactivity, environmental exposure, genetic and hormone replacement therapy, are associated with higher breast cancer risk (Veronesi *et al*., 2005; Howell *et al*., 2014). Generally, breast cancer risk factors could be defined as modifiable and non-modifiable factors. Lifestyle behaviors and exogenous hormone exposure are the examples of modifiable risk factors, whereas aging, family history, late-onset menopause and precancerous breast lesion are grouped in non-modifiable risk factors (Pruthi *et al*., 2015).

Genetic mutations, deficiency in diet and the use of oral contraceptives promote the chances of getting breast cancer (Veronesi *et al.*, 2005). Furthermore, the risk factors of breast cancer are mostly related to estrogen exposure and age, and mutations in BRCA1 or BRCA2 have been observed to increase the risk of getting breast cancer (Bozovic-Spasojevic *et al*., 2012). A woman, who has no family history of breast cancer, is considered to have average breast cancer risk. In general, women have the average 12% risk of developing breast cancer in the lifetime (Pruthi

et al., 2015). A woman is defined to have high breast cancer risk when her first-degree relatives are diagnosed with breast cancer before age 50 years old, or the women has history of atypical hyperplasia, history of lobular carcinoma in situ, increased mammographic breast density, 5 years Gail model risk of more than 1.7%, exposure to chest radiation at the age of 10 to 30 years and International Breast Cancer Intervention Study (IBIS) model lifetime risk of more than 20% (Pruthi *et al*., 2015). A woman, who has BRCA1 or BRCA2 mutation carrier, is defined at very high risk of developing breast cancer (Pruthi *et al*., 2015). It is estimated that 40-85% of women at very high breast cancer risk develop breast cancer in their lifetime. Reduction of calories uptake, more exercises, and lower consumption of alcohol help in reducing breast cancer formation (Parkin *et al*., 2011; Hansen & Steven, 2012).

2.5 Oral cancer

Oral cancer contributes 2-4% of cancer cases in the world (Markopoulos, 2012). Oral cancers, including cancer of tongue and mouth, are usually due to the uses of tobacco, alcohol and poor oral hygiene. A study showed that the risk of oral cancer is 100% related to tobacco and 90% related to alcohol, while 50.7% and 59.3% are related to sun exposure and older age, respectively (Lopez-jornet *et al.*, 2007). Besides tobacco and alcoholic drinks, diet, infectious agents, chemical irritants and frank carcinogens also contribute to oral cancers (Meurman, 2010).

South Asian countries have a higher rate of oral cancer because of the practices of bidi smoking, tobacco smoking, chewing betelquid and alcohol consumption (Madani *et al*., 2012; Zain, 2001). Bidi is the type of thin South Asian cigarette, which contains dried crushed tobacco flakes rolled in tendu leaves, whereas

betel quid is a type of tobacco that is wrapped inside a betel leave and plated on the side of the mouth (Madani *et al*., 2012). Smoking and quid chewing are carcinogenic because smoking and quid chewing cause oxidative stress, in which the acts damage DNA and lead to mutagenesis (Zain, 2001). Moreover, consumption of fast food, canned food, snack and fermented food increase the risk of getting oral cancer (Amtha *et al*., 2009). Fast food can cause oral cancer because of the high fat level content in the food. The fat contents in the fast food produce polycyclic aromatic hydrocarbons (PAH), which are proven to induce cancer in laboratory animals (Grrosvenor & Smolin, 2002). Furthermore, the fermented and canned food may contribute to cancer formation since these foods are associated with cancers especially gastric cancer (Grrosvenor & Smolin, 2002).

Current treatments for oral cancers include radiotherapy, surgery, chemotherapy or combination of these treatments (Huang & O'Sullivan, 2013). Oral cancer has higher mortality compared to other cancers because most of the patients, who are diagnosed with advanced oral cancer, cancer recurrences and metastasis, are difficult to be cured.

2.6 Oral squamous cell carcinoma (OSCC)

Oral squamous cell carcinomas (OSCC) often happen in the oral cavity and it is normally being ignored by patients during the early stage of cancer (Huang *et al*., 2014). The mortality rate of OSCC is not improved despite the advances of medical approaches (Markopoulos, 2012). The percentage of mortality of this disease in male patients is 3.1 out of 100,000 people, whereas the percentage of mortality in female patients is 1.4 out of 100,000 people (Mehrota & Yadav, 2006). The 5 year survival rate of OSCC is 40-50% (Markopoulos, 2012). Tongue, lips and floor of the mouse are the common sites for the development of OSCC. OSCC is difficult to be detected in the early stage of the disease because it is painless.

2.7 Current cancer treatments

Current anti-cancer drugs include bisphosphonates, camptothecin, topotecan, irinotecan and tamoxifen, which cause side effects to cancer patients namely hair loss, nausea, vomiting, fatigue and thinning (Jordan, 1993; Aslam *et al*., 2014). Tamoxifen is used to treat breast cancer. However, according to the study, tamoxifen causes liver tumors and endometrial carcinoma (Jordan, 1993). Other side effects of applying tamoxifen in cancer treatment are insomnia, vasomotor instability, headache, pain, nausea, vomiting and others (Jordan, 1993). The first line treatments for breast cancer that is currently practiced are surgery and radiotherapy (Liu *et al*., 2009). It is then supplemented with chemotherapy. ER-positive breast cancer cells can be halted by anti-hormonal drugs, in which the drugs inhibit the interaction of oestradiol with ER (Liu *et al*., 2009). However, ER-negative breast cancers are more invasive and aggressive with poorer prognoses (Bae *et al*., 2015; Rochefort *et al*., 2003;), which cannot be halted with anti-hormonal drugs. About 10% of breast

cancer incidence is also correlated to the genetic disorder, e.g. BRCA1 and BRCA2 mutations (Lillie *et al*., 2007).

Chemoprevention has been demonstrated to reduce cancer invasive especially in high-risk populations (Bozovic-Spasojevic *et al*., 2012; Howell *et al*., 2014). Chemoprevention is defined as the usage of natural or pharmacologic agents in reducing the invasiveness of cancers (Steward & Brown, 2013). Chemoprevention utilizes the selective receptor modulators, such as tamoxifen, and aromatase inhibitors, such as anastrozole, for reduction of breast cancer risk (Pruthi *et al*., 2015). Tamoxifen, raloxifene, exemestane and anastrozole are proven to reduce breast cancer incidence in women at increased risk of breast cancer (Pruthi *et al*., 2015). Generally, chemoprevention can be divided into three settings; primary, secondary and tertiary. Primary chemoprevention prevents the onset of disease, secondary chemoprevention cures the affected cell population and tertiary chemoprevention provides protection against second primary tumors (Bozovic-Spasojevic *et al*., 2012). The ideal chemoprevention agents must have the following requirements; effective against cancerous cells, safe and cost saving.

The mortality rate of OSCC remains low despite improvement in therapeutic approaches (Markopoulos, 2012). Surgery and radiotherapy are used in the early stage of oral cancer, as for the third and fourth stages of OSCC, a combination of surgery, chemotherapy and radiotherapy is used (Markopoulos, 2012). Targeted molecular therapy has limited adverse effects on non-cancerous cells and this approach has been applied to oral cancer patients.

2.8 Cancer cells resistance to chemotherapy

Chemotherapy removes cancer cells and reduces the growth of the cancer cells using drugs. However, standard chemotherapies do not inhibit the motility of the cancer cells (Ham *et al*., 2015). Chemotherapy induces programmed cell death and also inhibits division in cancer cells mainly (Ham *et al*., 2015). It is defined that chemotherapeutic failure was associated with genetic alteration of cancer cells and resistance to drug treatment (Rebucci & Michiels, 2013). Mutation in the genome of tumor cells and epigenetic changes contribute to the drug resistance. The resistances of cancer treatments do not limit to conventional chemotherapy, but also targeted therapies (Rebucci & Michiels, 2013). Cancer possesses thousands of mutations due to genome instability. A study defines six hallmarks of anti-cancer drugs resistance as following; alteration of drug targets, up regulation of the expression of drug pumps, detoxification, reduced apoptosis, alteration of cell proliferation and increased DNA damage repair (Cree & Charlton, 2017).

Cancer cells able to alter drug's targets through down regulation of targeted gene expression and activation of alternative molecular mechanisms (Cree & Charlton, 2017). In addition, the expression of drug efflux pumps is also important in developing resistance to drugs. The APT-binding cassette (ABC) superfamily of proteins in cancer cells protect the cells *via* pumping out of anti-cancer drugs (Cree & Charlton, 2017). MDR1 (ABCB1) gene is responsible for regulating the classical response to drug resistance. On the other hand, phenolic glycoprotein (PgP), a membrane-based xenobiotic pump molecule, able to eject drugs out from the cells, and hence develop resistance to anti-cancer drugs in the cells.

Expression of detoxification mechanism is one of the anti-cancer resistance hallmarks, whereby local drug detoxification contributes to anti-cancer resistance (Cree & Charlton, 2017). Reduced susceptibility to apoptosis and cell death, or avoidance of apoptosis also caused drug resistance (Cree & Charlton, 2017). Another hallmark of drugs resistance is the alteration of proliferation and increased the ability to DNA repair. When cancer drugs are introduced to cancer cells, many tumours are initially responding to the drug treatment, however, some of the cancerous cells might escape from death. The remaining cancerous cells populations may regrow and develop a resistance to cancer treatments (Cree & Charlton, 2017). Furthermore, cancer cells could develop also resistance to targeted therapies, but at a lower rate than conventional cancer drugs. For example, cancer cells compensate for EGFR blockade *via* other signaling pathways, such as amplification of MET (Cree & Charlton, 2017).

Taken together, multidrug resistance (MDR) is a system that protects cell population against drugs and other compounds (Liu, 2009). Adenosine triphosphate-binding cassette (ABC) transporter family and other transporter superfamilies (solute carrier transporters) play also the roles in anti-cancer drugs resistance, where ABC extrudes anti-cancer compounds out from cells, while solute carrier transporter mediates the cellular uptake of anti-cancer agents (Liu, 2009).

2.9 Camptothecin

Camptothecin is one of the important lead compounds in cancer research (Chu *et al*., 2014). It is a plant alkaloid that derived from the bark of *Camptotheca acuminate,* exerts anti-tumor activity against different types of cancer (Wall *et al*., 1996; Jones *et al*., 1997; Isah, 2016). Camptothecin could be also isolated from *Nothapodytes nimmoniana*, *Ophirrohiza mungos* and *M. foetida* (Premalakshmi *et al*., 2012). Camptothecin affects cell viability *via* breaking the double stranded DNA in S phase collision of replication forks with the topoisomerase 1-DNA complexes (Sims *et al*., 2009; Proszek *et al*., 2014). Camptothecins are quinoline alkaloids characterized by the planar pentacyclic ring system (Venditto & Simanek, 2010). Camptothecins lost its anti-cancer activity when the E-ring lactone in the structure is removed (Venditto & Simanek, 2010). Animal studies revealed that the toxic effects exerted by camptothecin, including emesis, diarrhea, dehydration and death (Venditto & Simanek, 2010). Furthermore, camptothecin demonstrated unpredictable toxicity in human in clinical trials.

Camptothecin is topoisomerase I inhibitor. There are two types of topoisomerases namely type I and type II topoisomerases. Topoisomerases induce transient DNA breaks *via* transesterification mechanism, where type I topoisomerase cleave and reseal one DNA strand, whereas type II topoisomerases cleave and reseal both DNA strands (Pommier *et al.*, 2010; Chu *et al*., 2014). Topoisomerase I is a nuclear enzyme that releases the topological stress (Proszek *et al*., 2014). It is often overexpressed in a variety of cancers. The enzymes are critically involved in DNA replication and transcription (Pommier *et al*., 2016). It is reported that the derivatives of camptothecin exert anti-cancer activity in lymphoma, colorectal and gastric

cancers (Chu *et al*., 2014). Major limitations of camptothecin including poor solubility and inactivity at physiological conditions. Various derivatives of camptothecin were developed in order to tackle the stability and solubility issues of camptothecin. Nevertheless, only 2 derivatives are approved for clinical use; irinotecan and topotecan (Venditto & Simanek, 2010). Topotecan is used for ovarian, small cell lung and cervical cancers, whereas irinotecan is used for metastatic colorectal cancer (Venditto & Simanek, 2010). Camptothecin was used as positive control in this study as camptothecin able to exert apoptotic cell death perfectly in breast cancer cell lines (Makin & Dive, 2001; Zeng *et al*., 2012).

2.10 Side effect of current cancer treatments

Side effects of cancer treatments range from vomiting, anxiety, pain, neuropsychologic impairment, hypertension and sexual dysfunction, where these side effects affect the quality life of cancer patients (Redd *et al*., 2001). Chemotherapy not only kills the cancer cells but it damages normal cells, as well (Aslam *et al*., 2014). A total 132 cancer chemotherapy drugs have been approved by the US Food and Drug administration (Aslam *et al*., 2014). According to the study, the most common side effects are the weakness, fatigue, hair loss, nausea and vomiting. Immunotherapy has significantly prolonged the survival time of patients with renal, melanoma, liver and lung cancers (Kirkwood *et al*., 2012; Yang *et al*., 2016), although the therapy causes also the side effects, such as fevers, diarrhea colitis, hepatitis, depression and hormone gland disorder (Yang *et al*., 2016). Surgery is a foundation of cancer treatment. It involves the removal of the entire tumor. The side effects of surgery include pain, limited range of motion, infection and bleeding (Stefani *et al*., 2017).

2.11 Metastasis

Metastasis is the main factor that promotes the diseases, e.g. cancers to claim over 6 million lives every year. Metastasis is the spread of cancer cells from one location to other distant location. Invasive cells of cancers can spread and invade other organs from the primary tumor. Metastasis involves complex cascade events, which allows tumor cells to detach from the primary site and attach to other organs. Cancer cells must be able to invade through extracellular matrix in order for metastasis to occur (Lee *et al*., 2008; Martin *et al*., 2013). The first step of metastasis is the detachment of cells primary tumors, cell extracellular matrix adhesion and proteolytic degradation of the matrix (Sato *et al.*, 2005; Martin *et al*., 2013). Metastasis is a crucial impediment for the successful cancer treatment; hence, understanding of the underlying mechanism in metastasis may lead to effective targeted therapy. If particular compounds have the ability to interrupt one or more of metastasis processes, the compounds have the potential to act as anti-metastasis therapy.

Most of the chemotherapy drugs were discovered from the study of potential anti-cancer agents in plants (Chanvorachote *et al*., 2016). More than 600 natural products display anti-cancer activity (Agbarya *et al*., 2014). However, inhibiting the anti-cancer activity alone is insufficient. There is increasing evidence revealed the activity of natural products in inhibiting cancer metastasis. The anti-metastatic agents, including curcumin, imperatorin (a compound isolated from *Angelica dahurica* root), and quercetin (Jemal *et al*., 2011; Nam *et al*., 2016; Youlden *et al*., 2008). Curcumin induces anoikis in lung cancer *via* down-regulation of Bcl-2 (Pongrakhananon *et al*., 2010), whereas imperatorin inhibits anchorage-independent growth of lung cancer

cells that also enhance anoikis response of lung cancer cells (Choochuay *et al*., 2013). Anoikis is a form of programmed cell death activated by the loss of cell-extracellular matrix that is recognised as a vital obstacle of cancer metastasis (Chanvorachote *et al*., 2016).

Epithelial-mesenchymal transition (EMT) is a process associated with cancer metastasis, where the epithelial cells are transformed into mesenchymal cells (Chanvorachote *et al*., 2016). EMT could be identified by the changes in cell morphology and expression of EMT markers, such as vimentin and N-cadherin. EMT alters cell components, such as adhesion molecules and cytoskeleton, thus the cells acquire high migratory ability to facilitate cancer metastasis. It is reported that natural products, such as 4,5,4'-trihydroxy-3,3'-dimethoxybibenzyl, isolated from Dendrobium ellipsophyllum inhibit EMT by reducing the level of EMT markers; Vimentin and Snail (Chaotham *et al*., 2014).

Angiogenesis is an important step in cancer metastasis, where the cancer cells develop new blood vessels. Natural products are also reported to act as the inhibitor of angiogenesis (Agbarya *et al*., 2014). Tumors regulate angiogenesis *via* secretions of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF). The secretions of those growth factors facilitate the growth of capillary and aid tumor growth by supplying nutrients and oxygen (Agbarya *et al*., 2014). Natural products, such as *Artemisia annua* (Chinese wormwood), *Viscum album* (European mistletoe), *Curcuma longa* (curcumin), resveratrol and proanthocyanidin (grape seed extract), quercetin and *Panax* ginseng have been demonstrated to display anti-angiogenesis activity in preclinical models (Agbarya *et al*., 2014).

2.12 Onions

Onions (*Allium cepa*) are found worldwide, including Europe, Asia, North America and Africa (Griffiths *et al*., 2002; Kwak *et al*., 2016). Onions are widely used as food and supplements to treat various diseases since ancient times (Lanzotti, 2006) because flavonoids and alk(en)yl cysteine sulphoxides (ACSOs) in onions are good for human health (Griffiths *et al.*, 2002; Kwak *et al*., 2016).

Anthocyanins and flavanols can also be found in onions (Griffiths *et al*., 2002; Kwak *et al*., 2016). According to Griffiths *et al*. (2002), onions have proven to exert anti-carcinogenic property, antibiotic activity, antioxidant property and anti-cancer activity. Flavonols in onion skin play important role in giving yellow, brown or red colour to the skin of the onion. Red onion contains a higher amount of anthocyanin and quercetin in the outer peel of onion (Griffiths *et al*., 2002). The outer skin of red onions had higher antioxidant activity than the skin of yellow onions and white onions. Similarly, red onion has higher phenol and total flavonoids contents than white and yellow onions (Shon *et al.*, 2004). Organosulfur compounds, such as diallyl sulphide in onions, contribute to anti-cancer property (Lai *et al*., 2013). It has been proven that flavonoids reduce free radicals or reactive oxygen species (ROS) produces in the biological system in the human body and also from external sources, such as smoking, radiations and pesticides that induce the development of cancer (Singh *et al*., 2009).

2.13 Red onion

Onion is consumed raw or cooked in our daily diet. Thiol compounds in onion play an inhibitory role of polyphenol oxidase (PPO) activity (Kim *et al*., 2005). *Allium* species are also shown to exert anti-bacterial, anti-fungal and antioxidant activities (Benkeblia, 2005). Approximately more than 500,000 tonnes of onion waste is discarded in the European Union (Waldron, 2001). The onion waste comprises of onion skin, roots and damaged bulbs (Benitez *et al*, 2011). The waste can be used for food ingredient and other applications as onions are potential in anti-cancer agents, antibiotics and anti-asthmatic (Moreno *et al*., 2006).

Two major subgroups of flavonoids; anthocyanins and quercetin can be found in onion. It is reported that different parts of onion contain the different type of quercetin. Quercetin 4'-glucoside and quercetin 3, 4'-diglucoside are mainly found in the flesh of onion, whereas quercetin aglycon is presented in higher concentration in onion skin (Downes *et al*., 2009; Downes *et al*., 2010). Quercetin is proven to act as antioxidant agent (Bonaccorsi *et al*., 2008). Moreover, alk(en)yl cysteine sulphoxides (ACSOs) in onion are also important in the health benefiting activity of onion (Benitez *et al*., 2011).

Allium sulphur compounds play a role in regulating antioxidant and apoptotic activities (Rose *et al*., 2005). According to Stajner *et al*. (2006), *Allium* species has health benefiting activity against tumor promotion and cardiovascular disease. Onions especially red onions have better radical scavenging activities than garlic. Onions exhibit various bioactive activities, such as anti-bacterial and anti-fungal (Skerget *et al*., 2009). It is proven that onion skin has a higher level of flavonoids