THE EFFECT OF *Oroxylum indicum* LEAVES METHANOLIC EXTRACT TOWARDS VCAM-1 EXPRESSION ON HeLa CELLS

by,

RASHIDI BIN DZUL KEFLEE

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In the name of Allah, the Most Gracious and Most Merciful

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LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMN

%	Percentage
±	Plus-minus sign
®	Registered sign
°C	Celsius
µl	Microliter
ATCC	American Type Culture Collection
CAM	Cellular adhesion molecules
cm	Centimetre
CO_2	Carbon Dioxide
CR	Conserved Region
DMEM	Dulbecco's modified Eagle's medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
E6AP	E6-associated protein
FACS	Flow cytometry
FADD	Fas-associated protein with death domain
FBS	Fetal Bovine Serum
FCS	Flow cytometry analysis

FDA	Food and Drug Administration
g	Gram
g	Gravity
G-CSF	Haemopoetic growth factors
HCL	Hydrochloric
HeLa	Human cervical cancer cell
Hep2	Human epithelial type 2
HGF	Human gingival fibroblast
HIV	Human Immunodeficiency Virus
HL-60	Human promyelocytic leukaemia cells
IC_{50}	Half maximal inhibitory concentration
IL-1β	Interleukin 1 beta
IL-6	Interleukin 6
IL-8	Interleukin 8
kbp	Kilo base pairs
L1	Major viral capsid
L2	Minor viral capsid
m	Meter
M	Molar
MBA	Methylene Blue Assay

MDCK	Madin-Darby canine kidney
mg/ml	Milligram per millilitre
NaCl	Sodium Chloride
NCI	National Cancer Institute
nm	Nanometre
OD	Optical Density
ORF	Open Reading Frame
p53	Tumour suppressor gene
PBS	Phosphate-Buffered Saline
pRb	Retinoblastoma tumour suppressor protein
PVs	Papillomaviruses
rpm	Round per minute
SEM	Standard error mean
STI	Sex transmitted infection
TNF-α	Tumour necrosis factor alpha
™	Trademark sign
URR	Upstream Regulatory Region
VCAM-1	Vascular adhesion molecules 1
VIA	Visual inspection with acetic acid
VLA-4	Very late antigen 4

α	Alpha
β	Beta
γ	Gamma
μg/ml	Microgram per millilitre
ν	Nu

ABSTRAK

Kanser serviks merupakan kanser yang keempat paling biasa dalam kalangan wanita, dan ketujuh dari keluruhan jenis kanser dengan anggaran sebanyak 528,000 kes baharu pada tahun 2012. Kanser serviks terletak di kedudukan yang ketiga dalam kalangan wanita yang berumur antara 15 hingga 44 tahun di Malaysia. *Oroxylum Indicum (O.indicum)* merupakan pokok daun luruh yang mempunyai kepentingan ekonomi, ekologi and perubatan yang tinggi. Tumbuhan ini juga dikenali sebagai pokok trompet india dan mudah dijumpai di kebanyakan negara tropika. Daun tumbuhan ini digunakan secara tradisi untuk rawatan sakit perut, angina dan juga kembung. VCAM-1 berinteraksi dengan ligan yang ekspres di atas permukaan sel endetelium vascular dan bertanggungjawab atas pengumpulan pelbagai sel darah putih ke dalam darah serta ekstravasasi ke dalam tisu. Kanser menggunakan mekanisme ini untuk membentuk kawasan tumor sekunder dan meningkatkan metastasis kanser. Tujuan kajian ini adalah untuk mengkaji kesan metanol ekstrak daun *O.indicum* (MEOIL) terhadap ekspresi VCAM-1 pada sel HeLa. Aktiviti anti-proliferatif secara *in-vitro* oleh MEOIL telah ditentukan melalui asai metilin biru untuk penentuan IC_{50}. Sel HeLa telah diransang dengan TNF-α selama sejam sebelum dirawat dengan MEOIL selama 24 jam. Penentuan ekpresi VCAM-1 telah dijalankan melalui analisis aliran sitometri (FCS) menggunakan pewarnaan permukaan. Ekstrak MEOIL menunjukkan kesan anti-proliferatif pada sel HeLa dengan IC_{50} 3.10 µg/ml, iaitu lebih rendah daripada cisplatin (kawalan positif) dengan IC_{50} 9.86 µg/ml. Ekspresi permukaan VCAM-1 dalam sel HeLa yang dirawat dengan MEOIL adalah menurun jika dibandingkan dengan sel HeLa yang tidak dirawat. Rawatan TNF-α dapat meningkatkan ekspresi VCAM-1 dan rawatan lanjut sel HeLa dengan MEOIL menunjukkan penurunan ekspresi VCAM-1. Kesimpulannya, MEOIL

boleh menurunkan ekspresi VCAM-1 dalam sel kanser serviks dan boleh mengurangkan metastasis kanser dan perkembangan kanser serviks.

ABSTRACT

Cervical cancer is the fourth most common cancer in women, and the seventh overall, with an estimated 528,000 new cases in 2012. Cervical cancer ranks as the third female cancer in among women aged 15 to 44 years in Malaysia. *Oroxylum Indicum* (*O.indicum*) is a medium-sized deciduous tree with high economical, ecological and medicinal importance. This plant which also known as Indian trumpet tree can easily be found in many tropical countries. The leaves are traditionally used as stomachic, carminative and flatulent. VCAM-1 interact with the ligands expressed on the surface of the vascular endothelial cells and responsible for selective recruitment of different leucocyte into the blood and extravasation to the tissue. Cancer utilize these mechanism to form secondary tumor site and increase metastases of cancer. The aim of this study is to examine the effect of *O.indicum* leaves methanolic extract (MEOIL) towards VCAM-1 expression on HeLa cells. *In vitro* anti-proliferative activity of MEOIL extract towards HeLa cells was determined by Methylene Blue Assay (MBA) for IC_{50} determination. HeLa cells were stimulated with TNF-α for one hour, prior to 24 hours treatment with MEOIL. Determination of VCAM-1 expression was conducted *via* flow cytometry analysis (FCS) by surface staining. MEOIL extract shows the anti-proliferative effects on HeLa cells with IC_{50} of 3.10 µg/ml, which is lower than cisplatin (positive control) with IC_{50} of 9.86 µg/ml. The surface expression of VCAM-1 is decreased in MEOIL-treated HeLa cells compared to untreated cells. TNF-α treatment was able to increase the expression of VCAM-1 and further treatment of HeLa cells with MEOIL showed a decreased expression of VCAM-1. In conclusion, the MEOIL could down-regulate the VCAM-1 expression in cervical cancer cell which could reduce the metastases of cancer and decrease the progression of cervical cancer.

CHAPTER 1

INTRODUCTION

Highly diverse DNA viruses, papillomaviruses (PVs) are ubiquitous and their origin exist within the existence of modern humans (Herbst et al., 2009; zur Hausen, 2009). PVs are also described as "types" and tremendously studied in humans where almost 100 human PVs (HPVs) types were found and described based on the isolation of complete genomes (De Villiers et al., 2004). Benign tumours such as warts and papilloma are commonly occurred in their natural host and related species due to PVs. Papilloma are induced in the skin and mucosal epithelia and generally occurred at specific sites of the body. The specific high risk PVs could induce some papillomatous proliferation which lead to malignant progression (Clifford et al., 2003).

Hypothesis made by Dr. Rigoni-Stern, the Italian physician in 1842 stated that cervical cancer might be connected with sexual behaviour (Gaspani & Panatto, 2009). The number of cervical cancer cases were higher in married women and prostitutes than virgins and this suggests that the transmission maybe due to sexually activity. The high risk HPV types account for etiological agents of cervical cancer. In 2006, Food and Drug Administration (FDA) has approved the HPV vaccine to prevent cervix cancer (Ramirez-Fort, et al., 2014).

At least 13 types of HPV could cause cancer from over 100 types of HPV which also known as high risk type. Generally, 70% of cervical cancers and precancerous cervical lesions caused by two HPV types namely HPV 16 and HPV 18 (Dunn & Tan, 2010). Approximately 530,000 new cases were reported in 2012, and with that, cervical cancer became the 4th most frequent cancer in women worldwide (Domingo et al., 2008). Cervical cancer ranks as the 2nd most cancers affecting women who are living in less developed regions. In low and middle income region, there were approximately 270,000 women died from cervical cancer in 2012. In Malaysia, approximately 2145 new cervical cases were diagnosed and account for the second most female cancer in women aged 15 to 44 in year 2012. These made the cervical cancer as the second most common female cancer behind the leading most cancer which is breast cancer in the same year (Human Papillomavirus and Related Diseases Report, 2015).

HPV vaccination program is a national program in Malaysia. HPV vaccination routine immunisation programme begin in 2010 and progressively continue for the next consequent years. Women are advised to take the vaccination at the age of 13 for further prevention of HPV. These prevention measure has led to successful coverage of 87% in 2011 of full course HPV vaccination coverage for routine immunization. FDA licensed two HPV vaccines namely Cervarix and Gardasil. Cervarix is a bivalent HPV vaccine which is important for preventing two HPV types, 16 and 18. HPV 16 and HPV 18 account for 70% of cervical cancer. However, this vaccine gives adverse effects such as mild injection site reactions (pain, swelling, erythema, and pruritus), fatigue, headache, myalgia and gastroenteritis (Yanofsky, Patel, & Goldenberg, 2012). Gardasil is a quadrivalent HPV vaccine which prevents four types of HPVs; HPV 16, 18, 6 and 11. HPV 6 and 11 are responsible for 90% of genital warts.

The best way in combating a patient with an extensive cervical cancer is by chemotherapy. The disadvantages of this strategy commonly related to their lack of selectivity and specificity. The chemotherapeutic drugs also exhibit numerous side effects to the patient which may lead to the secondary infection (Zazali, Abdullah, & Izani, 2013). There were different therapies with significant in term of cost, duration of therapy, dosing schedules and adverse effects. The therapies may be as topical, surgical or immunomodulatory. As of yet, there is little evidence to suggest that one class of treatments is not more effective than another nor has a single therapy emerged as the gold standard for treatment (Yanofsky et al., 2012).

Both western and Asian cultures use the medicinal plants which act as important agents in traditional medicine and source material for the production of dietary supplements. Bioactive compounds from natural plants also could serve as prototypes for production and synthesis of new drugs which may have same biological and therapeutic activities (Sponchiado et al., 2015). The examples of medicinal plants used in anti-cancer therapy are *Origanum dayi (O.dayi)*, *Artemisia monosperma (A.monosperma)*, *Urtica dioica (U.dioica)* and *Oroxylum indicum (O.indicum)*. *O.dayi* has been associated with suppression of growth in human leukaemia Molt 4B and HL-60 cell lines. *A.monosperma* exhibit anti-cancer properties against colorectal and breast cancer (Solowey et al., 2014). *U.dioica* also shows anti-tumour activity against human prostate cancer cells (Konrad et al., 2000).

Oroxylum indicum leaves or beko leaves is common in Malaysia that usually serve as 'ulam' or salad. The tree usually grows in Asian tropical as well as subtropical low altitude forest and cultivated on a road sides and slopes. The tree is also known as'midnight horror' due to its powerful stink smell from the flower at night which is crucial in attracting the bats for pollination (Dinda et al., 2015). The leaves could be a potential as a new anti-cancer therapy as the leaves were accentuated for anticancer studies. The anti-cancer properties of *O.indicum* includes anti-proliferative effects on Hep2 cell lines by ethanol extract of *O.indicum* and methanolic extract of this plant inhibits the mutagenicity of Trp-P-1 in an Ames test (Factor et al., 2014). Methanolic extract of *Oroxylum indicum* leaves (MEOIL) shows inhibition in proliferation of HeLa cell *in-vitro* (Zazali, Abdullah, & Izani, 2013).

However, none of the previous studies on *O.indicum* as the herbs of interest specifically conducted on its effects on the adhesion molecules vascular adhesion molecules 1 (VCAM-1) that are commonly expressed during cervical cancer. Hence, this study is specifically focus on the effect of methanol extract of *O. indicum* leaves methanol extract on the expression of vascular cellular adhesion molecules-1 (VCAM-1) in cervical cancer cells (HeLa cells).

1.1 Hypothesis

Oroxylum indicum (O.indicum) leaves could down-regulate the expression of adhesion molecules vascular cellular adhesion molecules (VCAM-1) in cervical cancer cells (HeLa cells).

1.2 Objective

General Objective;

To examine the effect of *Oroxylum indicum* leaves methanolic extract towards VCAM-1 expression on HeLa cells.

Specific Objectives;

i. To prepare the methanol extract of *O.indicum* leaves (MEOIL).

ii. To determine the anti-proliferative activity of *O.indicum* extract & cisplatin against HeLa cells *via* Methylene Blue Assay (MBA).

iii. To ascertain the expression of VCAM-1 in HeLa cell treated with *O.indicum* extract by Flow cytometry.

1.3 Rationale of study

Chemotherapy has many side effects due to the lack of specificity and sensitivity. Cervical cells proliferates non-stop due to HPV infection. However, the normal tissue homeostasis could be regulated in the presence of potential apoptotic inducer. *O.indicum* was found to act as apoptosis inducer and act selectively against cancer cell without affecting normal cells proliferation (Zazali, Hasmah, & Izani, 2013). The information on expression of adhesion molecules by apoptosis-inducing activities of *O.indicum* are still poorly understood. Hence, this study was done to ascertain the expression of VCAM-1 on HeLa cells treated with *O.indicum*. This is important to improve the fundamental understanding of *O.indicum* mechanism as potential chemotherapy agent.

CHAPTER 2

LITERATURE REVIEW

2.1 The epidemiology of Human Papillomavirus (HPV)

There are approximately 528,000 new HPV cases worldwide reported in 2012 which 0.38% decreased from cases that were reported in 2008. Moreover, the number of death in 2008 was 275,000 and ranks as the fourth most common cause of cancer death in 2012 (Human Papillomavirus and Related Diseases Report, 2015). Based on figure 2.1, Malaysia ranks as the fourth highest among South-East Asia countries in the corpus uteri cancer cases among females attribute to excess body mass index in 2012 with 237 cases (Ferlay J et.al, 2013).

There was 2145 new cervical cancer cases were diagnosed in Malaysia which account for 0.41% of worldwide annual number of new cases in 2012 (Release, 2013). Based on figure 2.2, the crude incidence rate is 14.8 per 100,000 women per year and the age-standardized incidence rate in Malaysia is 15.6 per 100,000 women per year. This cancer ranks as the 2nd cause and most common female cancer in women aged 15 to 44 years in Malaysia after the breast cancer in the same year. The annual number of deaths in this country is 621 which account for 0.23% from worldwide annual number of deaths. The crude mortality rate is 4.3 per 100,000 women per year which put this cancer as the 5th cause of female deaths in Malaysia behind ovary, colorectal, lung and breast cancer (Human Papillomavirus and Related Diseases Report, 2015).

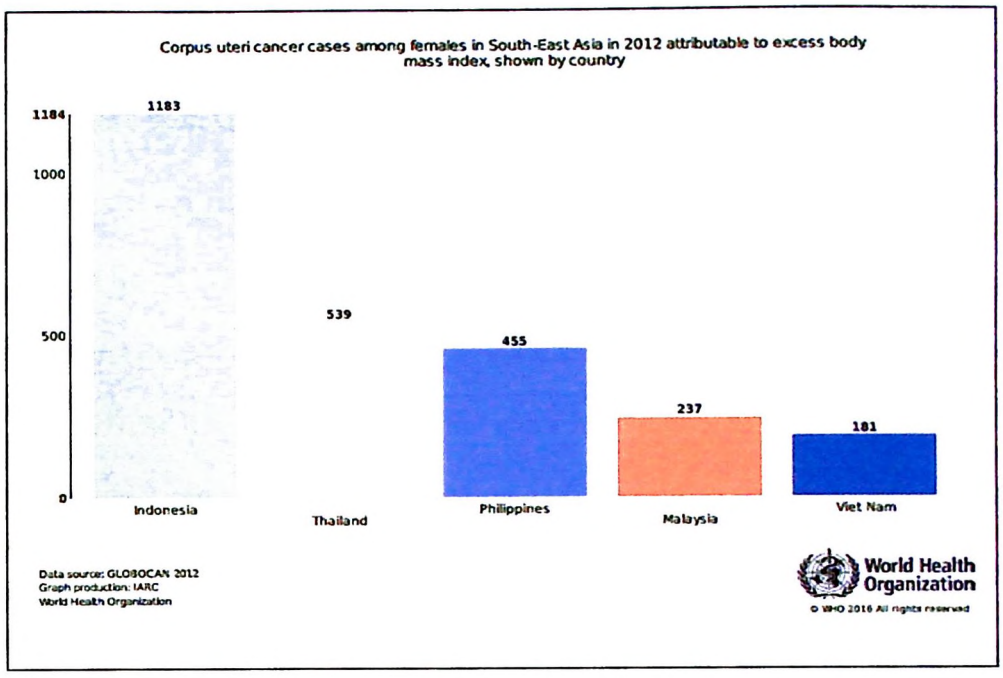


Figure 2.1: The corpus uteri cancer cases among females in South-East Asia in 2012 attribute to excess body mass index, shown by country. Adapted from Ferlay J et.al (2013)

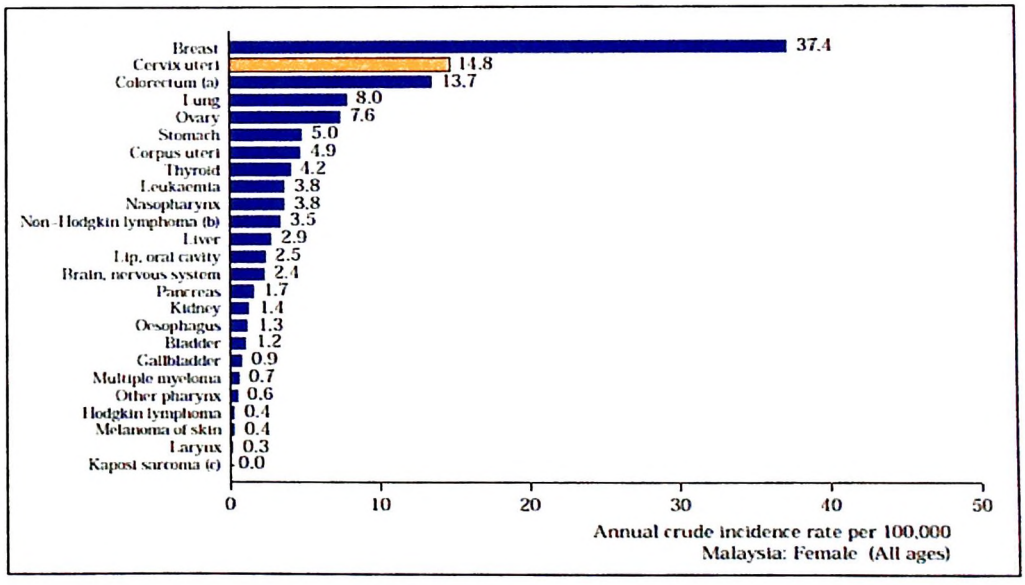


Figure 2.2: Age-specific cervical cancer incidence compared to age-specific incidence of other cancers among women 15-44 years of age in Malaysia (estimations for 2012). Adapted from Human Papillomavirus and Related Diseases Report (2015).

2.2 The biology of HPV

2.2.1 Virion and Genome Structure

The HPVs belong to the Papovaviridae family. The capsid is icosahedral in shape, with no envelope. The DNA molecule is closed with 8 kilo base pairs (kbp) double-stranded which comprises early and late genes clustered in separate regions (Fakhraei & Haghshenas, 2013). This naked icosahedral capsid has diameter of 55 nm and composed of 72 pentameric capsomeres. The DNA strand contain the genetic information and consist of at least eight open reading frames (ORF) (Orth, 1999). Based on Figure 2.3, there were three parts in viral genome which are noncoding region, early region and late region. The proteins encoded by early genes involve in viral DNA replication, transcription and cellular transformation. The major viral capsid (L1) and a minor capsid protein (L2) were encoded by late gene. Upstream regulatory region (URR) also known as the long control region which situated between these two regions. The promoters and elements involved in DNA replication and transcription situated in the noncoding region (Tyring, 2000).

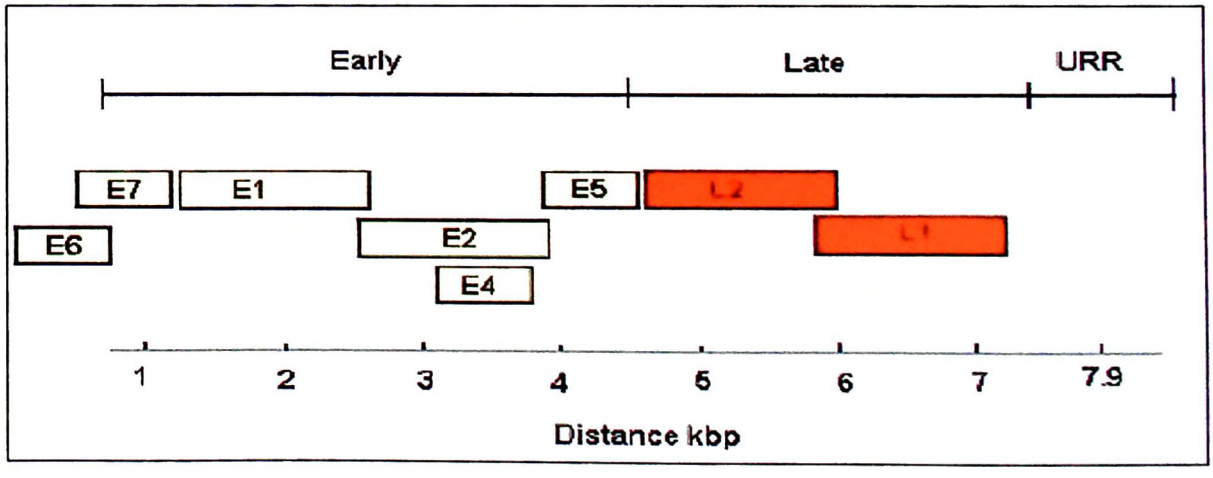
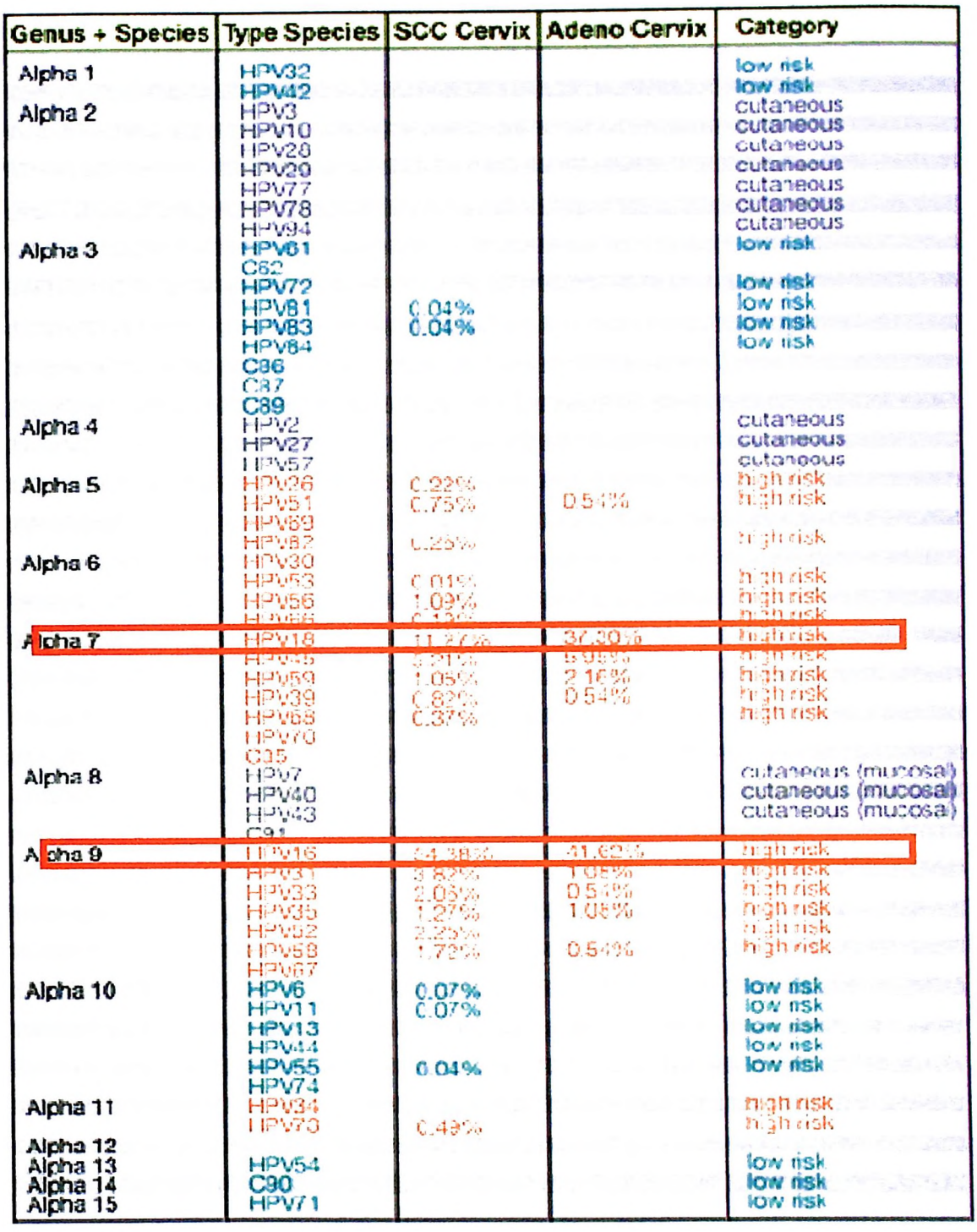


Figure 2.3: A schematic representation of linear HPV16. Adapted from Raybould, (2011).

2.2.2 Types of HPV

In 1995, the International Papillomavirus Workshop agreed that if there was more than 10% dissimilarity in the L1 region of the HPVs genome, the HPVs would be considered to be different from each other. This is because the L1 region has a highly conserved gene. There are 5 different genera in HPVs which are α, β, μ, γ and ν (Sampogna et al., 2012). In human's body, the papillomaviruses emerged from point mutations in various locations of the viral genome. This is because the role of gene in displaying a major homology among HPVs which lead to discovery of sequenced 150 HPV types. The types of HPV in clinical manifestation can be classified into preferentially infect cutaneous tissue and generally occupy mucosal surfaces (Doorbar, 2006). Figure 2.4 shows alpha (α) genes consist of low risk HPV types and high risk HPV types. HPV type 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59 are considered as high-risk cancer-causing types. HPV type 2, 3, 10, 57, 6, and 11 are the low-risk HPVs (Doorbar, 2006 and Tyring, 2000). The high-risk HPVs such as HPV 16 and 18 can cause cervical carcinoma (Fakhraei & Haghshenas, 2013).



Genus + Species	Type Species	SCC Cervix	Adeno Cervix	Category
Alpha 1	HPV32			low risk
	HPV42			low risk
Alpha 2	HPV3			cutaneous
	HPV10			cutaneous
	HPV28			cutaneous
	HPV29			cutaneous
	HPV77			cutaneous
	HPV78			cutaneous
	HPV94			cutaneous
Alpha 3	HPV61			low risk
	C62			
	HPV72			low risk
	HPV81	0.04%		low risk
	HPV83	0.04%		low risk
	HPV84			low risk
	C86			
	C87			
	C89			
Alpha 4	HPV2			cutaneous
	HPV27			cutaneous
	HPV57			cutaneous
Alpha 5	HPV26	0.22%		high risk
	HPV51	0.75%	0.54%	high risk
	HPV69			
	HPV82	0.25%		high risk
Alpha 6	HPV30			
	HPV53	0.01%		high risk
	HPV56	1.09%		high risk
	HPV66	0.12%		high risk
Alpha 7	HPV18	11.27%	36.39%	high risk
	HPV45	3.21%	5.95%	high risk
	HPV59	1.05%	2.16%	high risk
	HPV39	0.82%	0.54%	high risk
	HPV68	0.37%		high risk
	HPV70			
	C35			
Alpha 8	HPV7			cutaneous (mucosal)
	HPV40			cutaneous (mucosal)
	HPV43			cutaneous (mucosal)
	C91			
Alpha 9	HPV16	54.38%	41.62%	high risk
	HPV31	3.82%	1.08%	high risk
	HPV33	2.05%	0.54%	high risk
	HPV35	1.27%	1.08%	high risk
	HPV52	2.25%		high risk
	HPV58	1.72%	0.54%	high risk
	HPV67			
Alpha 10	HPV6	0.07%		low risk
	HPV11	0.07%		low risk
	HPV13			low risk
	HPV44			low risk
	HPV55	0.04%		low risk
	HPV74			
Alpha 11	HPV34			high risk
	HPV73	0.49%		high risk
Alpha 12				
Alpha 13	HPV54			low risk
Alpha 14	C90			low risk
Alpha 15	HPV71			low risk

Figure 2.4: Association of alpha papillomavirus types with cervical cancer. Adapted from Doorbar (2006).

2.3 Transmission & risk factor of HPV-associated cervical cancer.

The risk of having cervical cancer among women increase when their partner is positive with HPV. Furthermore, the risk of cervical cancer is also increased with the presence of co-factor such as long-term oral contraceptives which exceeds five or more years, high parity, cigarette smoking, co-infection with human immunodeficiency virus (HIV) or co-infections with other sex transmission infections (STIs) (Chelimo et al., 2013). The increased number of smoked per day may increase the risk for cervical cancer (Fonseca-Moutinho, 2011). Previous study was done on HPV DNA positive women showed that the risk for development of cervical cancer was associated with smoking intensity. Furthermore, the HPV-positive women who has seropositive for *Chlamydia trachomatis (C.trachomatis)* antibodies also at risk of having invasive cervical cancer (Hildesheim et al., 2001).

The behaviours related to sexual activity is the major risk factors of HPV transmission. Previous study has shown that very low HPV prevalence in females who have not initiated sexual activity (Brown et al., 2008). However, highest risk for infection with HPV 16 were recorded in women with new partners in the previous 5 to 8 months. One of the important risk factors for HPV transmission is the number of lifetime sexual partners among females. This is because with an increasing number of lifetime sexual partners would increase the detection of HPV DNA in both males and females. In addition, the age differences between women and her partner shown to be a risk factor for HPV transmission. This is because an older male partner would high chances of being HPV carriers. The usage of oral contraceptive use for a long-term has also been associated with an increased risk for HPV (Hildesheim et al., 2001).

2.4 HPV E6 and E7 oncoproteins

The expression of E6 and E7 proteins were focused in early studies of HPV viral oncogenes. The degradation of cellular tumour suppressor gene, p53 and modulation of retinoblastoma tumour suppressor protein (pRb) are the important mechanism associated with HPV oncoproteins. The E6 protein was shown to interfere with p53, while modulate the retinoblastoma tumour suppressor protein (Yim & Park, 2005). Besides that, cellular transformation by new mechanism including genomic stability, morphology and shape of the cell and angiogenesis regulation were found to be due to the viral oncoproteins (Tyring et al., 2000).

Figure 2.5 shows high risk HPV E6 proteins target the cellular E3 ubiquitin ligase E6AP to p53, resulting in the transfer of ubiquitin peptides from E6AP to p53, which marks p53 for degradation by the 26S proteasome. E6-stimulated degradation interferes with such biological functions of p53 which then perturbing the control of cell cycle progression, leading finally to increased tumour cell growth (Munger & Halpern, 1997). Another study showed that E6 interacts with and inactivates the proapoptotic Bcl-2 family proteins Bax and Bak, and apoptotic mediators FADD (Fas-associated protein with death domain), procaspase 8 and c-myc genes (Lechner & Laimins, 1994).

Molecular weight protein of E7 oncogene is approximately 100 amino acids. There were different part in E7 proteins which are the three conserved regions (CR), the NH2-terminal CR1 domain, the CR2 region and the COOH-terminal CR3 domain. The NH2-terminal CR1 domain does not directly contribute to pRb binding but necessary for cellular transformation and pRb degradation (Munger, Howley, & Di Maio, 2007).

Based on Figure 2.5, HPV E7 proteins interact with the retinoblastoma protein pRb, which are negative cell-cycle regulators involved in the G1/S and G2/M transitions. The interaction between high-risk E7 and pRb results in enhanced phosphorylation and degradation. pRb destruction leads to the release of E2F family of transcription factors and the subsequent activation of genes promoting cell proliferation (Yim & Park, 2005).

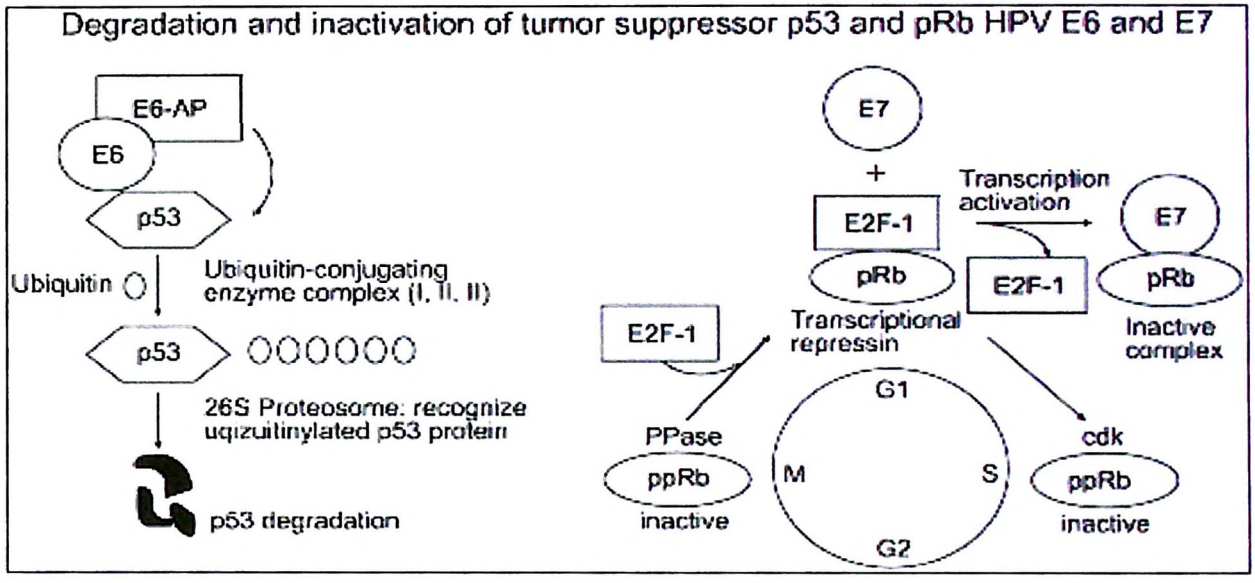


Figure 2.5: HPV E6 and E7 binding to P53, with pRb interfering with the normal functions of the tumour suppressors. Yim & Park (2005).

2.5 Roles of adhesion molecules in metastasis of cancer

Cell adhesion molecules (CAM) such as vascular cellular adhesion molecules (VCAM-1) play key roles in various stages of tumour angiogenesis, tumour progression and metastasis. VCAM-1 is a 110 kDa glycoprotein which belongs to the immunoglobulin superfamily group of adhesion molecules which commonly expressed on tissue macrophage, dendritic cells, epithelial cell and stimulated endothelial cells (Ding et al., 2003). VCAM-1 is an endothelial adhesion molecule which was described as a cytokine-inducible molecule (Collins et al., 1995). One of HPV16 oncoproteins known as E7 is strongly induce the expression of VCAM-1 in cervical microvascular endothelial cells (D'Anna et al., 2001). The leucocytes were recruited to the sites of inflammation by binding of VCAM-1 to leucocyte integrin VLA-4 (very late antigen-4) (Lee et.al, 2001).

Adhesion molecules are also important in the pathogenesis of inflammatory disease such as arthritis (Szekanecz & Koch, 2000). Up-regulation in response of pro-inflammatory cytokines and their role as co-stimulatory receptors in the activation of inflammatory cells also the key role of adhesion molecules in the inflammatory pathogenesis (Pigott et al., 1992). Besides that, adhesion molecules also important in vast of cellular function which includes signal transduction, cellular communication and recognition, embryogenesis, inflammatory responses and apoptosis (Okegawa et al., 2004). On the surface of human gingival fibroblast (HGF), up-regulation of adhesion molecules such as VCAM-1 plays an important role in infiltration and retention of inflammatory cells at sites of periodontal diseased tissue. The released of pro-

inflammatory cytokines such as IL-1β and TNF-α were important in relation with the progression of periodontal disease (Hosokawa et al., 2006).

Tumour growth metastasis, immunosuppression and angiogenesis would occur if the expression of cytokine is out of control. These cytokines such as IL-1β and TNF-α can be up-regulated during inflammation and wound repair as well as not cancer specific (Henderson et al., 2016). Previous study has shown that up-regulation of adhesion molecules by cytokines which are produced by tumour cells were detected in the supernatants of TNF-activated endothelial cells and also in the culture media of colonic carcinoma cell lines (Pigott et al., 1992).

The effect of VCAM-1 towards human health is a great concern due to its ability cause angiogenesis which lead tumour progress (Collins et al., 1995). HPV 16 E6 & E7 proteins led to increase in VEGF and IL-8 expression, proteins involved in regulating the angiogenic signalling in cancer cells. VEGF induces an increase in cervical epithelial sheet fold which moderate increase in cervical tissue oedema. This lead to increase level of VCAM-1. The tumour would be able to received continuous nutrients, gas exchange and waste disposal from angiogenesis. However, the abnormal adhesion molecules expression contributes to tumour angiogenesis. Neoplastic cells were stimulated by tumour angiogenesis to produce growth factors specific for endothelial cells. This mechanism leads the neoplastic cells to grow inside the host's blood vessels. Previous study has shown that VCAM-1 contributed in tumour angiogenesis and has been confirmed to be linked to tumour progression, haematogenous metastasis and tumour recurrence (Ding et al., 2003).

2.6 Treatment & prevention of cervical cancer

The aim in cervical cancer treatment involves chemotherapy, chemotherapy with addition to another main treatment such as surgery called adjuvant chemotherapy. Before the surgery or radiotherapy, the chemotherapy usually was given to the patient to enhance the effectiveness of the treatment. This process also known as neoadjuvant chemotherapy. However, the cytotoxic medicines are too powerful to attack cancer cells which lead to unwanted side-effects such as fatigue and blood disorders to the patient. This is because the cytotoxic medicine could affect the normal dividing cells. Besides giving the bad side-effects to the patient, chemotherapy are very costly towards low income patient (Jacobo-Herrera et al., 2016). Furthermore, chemotherapeutic drugs in the cancer treatment also shows lack of sensitivity and specificity towards the cancer cells which cause side effects towards patient health (Zazali, Abdullah, & Izani, 2013).

Cervical cancer incidence and mortality can be reduced with cervical screening programmes. In coming decades, the burden of cervical cancer would be effectively reduced due to introduction of HPV vaccination across the globe (Human Papillomavirus and Related Diseases Report, 2015). Population-based programmes are one of the strategies which implemented in some countries. These includes in target populations, each round of screening women are individually identified and invited to attend screening. Another example is opportunistic screening where the individual has a right to make a decision to do the screening or on encounters with health-care providers. Cytology is the most frequent method for cervical cancer screening. The alternative methods such as HPV DNA tests and visual inspection with acetic acid (VIA) are also screening method. In low-resource settings, VIA is an alternative to cytology-based screening. In

some countries, HPV DNA testing is used as a primary screening which then followed by more specific test such as cytology (Curt et al., 2000).

It was reported almost 34.8 new cases of cervical cancer per 100,000 women were diagnosed and 22.5 per 100,000 women die annually in sub-Saharan Africa. The huge differences were elucidated when compared to the North America when they only diagnosed 6.6 new cases per 100,000 women and 2.5 per 100,000 women died. Lack of access to effective screening and to services that facilitate early detection and treatment would cause the huge differences in those figures between sub-Saharan Africa and North America (Release, 2013).

HPV vaccination is important to protect an individual from getting HPV infections that may cause cancer. The vaccination is given in a series of shots in several months (Them, 2014). Cervarix and Gardasil are two HPV vaccines which are licensed by the FDA (Schwartz, 2010). HPV types 16 and 18 are two types of HPV which are prevented by Cervarix. These types account for 70% of cervical cancers (Einstein et al., 2009). The quadrivalent HPV vaccine (Gardasil) prevents HPV 6 and 11, which cause 90% of genital wart (Donovan et al., 2011). Gardasil also gives protection against cancers of the anus, vagina and vulva it is the only vaccine that can be used for males (Human Papillomavirus and Related Diseases Report, 2015).

Unfortunately, Gardasil and Cervarix shows no therapeutic effect against pre-existing HPV infection. HPV vaccines is infective in targeting L1 and/or L2 in elimination of pre-existing infection. This is because infected basal epithelial cells and cervical cancer cells do not express detectable levels of capsid antigen (L1 and/or L2). Furthermore, both Cervarix and Gardasil are highly cost vaccine which were not available in low-resource area (Monie et al., 2008).

2.7 Alternative medicine with medicinal plant

In both developing and developed countries, the use of medicinal plants in humans was widespread and these plants also have a long history of use. The treatment of disease which is obstinate and incurable in other systems of medicine can be replaced with herbal medicines. The lower side effects, better patient tolerance, cost effective and acceptance due to long history of use are the main components that increase the popularity of these herbal medicines (Vermani & Garg, 2002).

The usage of medicinal plant in research commonly involves inflammations-disease related, cancer & tumour, myocardial ischemia, circulation disorders, hypertension, liver injuries and toxicity, diabetes-related complications, haematological, biochemistry and histopathology, immune-related diseases, gastro-related diseases, quality of life among healthy adult and also behaviour studies (Prakash et al., 2013). Furthermore, various types of bioactive compounds have been isolated which is used as a source of medicines and were characterised as therapeutic agents. Surprisingly, a synthetic favopiridol which is derived from the plant alkaloid rohitukine is isolated from *Dysoxylum binectariferum* was subjected to clinical studies under phase I and II clinical trials. This bioactive compound has broad activity against tumours, leukaemia, lymphomas and solid tumours (Christian et al., 1997).

Hypericum perforatum and *Taraxacum* were few of the medicinal plant which have been used in anti-cancer therapy. The active compound hypericin from *Hypericum perforatum* has ability to reduce and retarded the glioma cell lines growth in vitro. This active constituent also was linked to inhibition of protein kinase-C in induction of glioma

cell death. This plant exhibit greater inhibit glioma cells activity when compared to tamoxifen (Alecu et al., 1998).

In addition, *Taraxacum officinale* is also known as dandelions. Several tribes and countries commonly use this plant to against cancer. This species was investigated against tumour progression which related to invasion and proliferation. Moreover, the growth of breast cancer cells were reduced by treatment with leaf extract of *Taraxacum officinale* (Sigstedt et al., 2008).

2.8 *Oroxylum indicum*

Oroxylum indicum is a scientific name which is synonym with *Bignonia indica*, *Spathodea indica*, *Calosanthes indica* and *Hippoxylon indica* and its botanical classification of Kingdom, *Plantae*; Class, *Magnoliophyta*; Order, *Lamiales*; Family, *Bignoniaceae*; Genus, *Oroxylum* and Species, *Indicum* (Neelu, Alok, & Tapan, 2014). Previous study used *Oroxylum indicum* species as a plant of interest in anti-cancer therapy (Zazali, Abdullah & Izani, 2013). These ethno medicinally important tree have active principles for their anti-cancerous, anti-inflammatory, anti-helminthic, anti-bronchitis, anti-leucodermatic, anti-rheumatic, anti-anorexic and analgesic properties which mainly in root bark (Factor et al., 2014).

O.indicum consists of small or medium sizes can grow up to 12 m with soft light brown or greyish brown bark as well as corky lenticels (Figure 2.6). It is 90 to 180 cm long with 2 to 3 pinnate with 5 or more pairs of primary pinnae, swollen at the junction of branches. There are 2 to 4 pair leaflets with ovate or elliptic and the large leaf stalks wither and fall off the tree and collect near the base of the trunk. These would appear like a pile of broken limb bones. The outside of the flowers are reddish purple and pinkish-yellow inside. The flowers are numerous and bloom at night. These flowers emit strong and stinky odour which attracts bats (Deka et al., 2013 ; Factor et al., 2014).

In Ayurvedic and folk medicine, *O.indicum* has been extensively used in their practice. Malaysian communities believes that this plant can cure toothache, wound, splenomegaly, dysentery, cholera, loss of appetite and fever. Moreover, any part of the plant may be used for making a decoction for external uses in childbirth. Furthermore,

decoction of leaf of this plant can treat rheumatic pain, splenomegaly, ulcer, cough and bronchitis (Neelu et al., 2014).

Figure 2.6: Beko tree or *O.indicum* tree

Numerous of secondary metabolites has been found in this plant includes glycosides, alkaloids, tannins & terpenoids (Neelu et al., 2014). The flavonoids such as chrysin, oroxylin-A, scutellarin and baicalein also found in the leaves of this plant. Moreover, *O.indicum* leaves are also found to contain quercetin-3-o-α-L-arabinopyranoside, 1-(2-hydroxyethyl) cyclohexane-1, 4-diol and apigenin18 (Ahad et al., 2012). It has been reported that the seeds of *O.indicum* contain four flavonoids identified as chrysin, baicalein, baicalein-7-0-glucoside, baiaclein 7-o-di glucoside which inhibit proliferation of cancer cell line *via* induction of apoptosis (Masila et al., 2015).

A methanolic extract of the fruits of *Oroxylum indicum*, which is widely used in traditional Chinese herbal medicine for its anti-inflammatory, anti-pyretic and anti-hypersensitivity effects, inhibited in vitro proliferation of HL-60 cells. The flavonoid baicalein was found as an active component in the extract (Roy et al., 2007). Previous study suggested that MEOIL has successfully exerted a selective anti-proliferative effect on HeLa cells. Thus, MEOIL is much less cytotoxic than cisplatin (Zazali, Abdullah, & Izani, 2013).

The anti-cancerous properties of root bark *O.indicum* was shown on CEM, B-16 and HCT-8 cell lines. Petroleum ether hot extract of *O.indicum* could induce apoptosis in breast cancer by effectively target Estrogen Receptor (ER) - negative breast cancer cells without harming the normal cells (Naveen Kumar et al. 2012). Ethanolic extract of *O.indicum* was found to have anti-proliferative against Hep-2 cell lines (Nakahara et al., 2001).