

CHAPTER 1

INTRODUCTION

Cancer is the leading cause of death in human population worldwide (Reddey *et al.*, 2003). Cervical cancer is the second most common cancer in Malaysia (WHO, 2007) and fourth most common cause of women death in Malaysia (The Second Reports of National Cancer Registry, 2004). Other researcher found that cervical cancer ranks third of all cancers in Malaysia (Norhayati, 2003).

The most important risk factor in the development of cervical cancer is infection with a high-risk strain (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) of [human papillomavirus](#). Types 16 and 18 are generally acknowledged to cause about 70% of cervical cancer cases (Munoz *et al.*, 2003). HPV 16 and 18 are two most common HPV types in Southeast Asia (WHO, 2007); although HPV 18 alone is relatively more frequent compared to the type distribution estimates in the rest of the world (Smith *et al.*, 2007; Sriamporn *et al.*, 2006).

The carcinogenic process has now been extensively investigated in terms of both biological and molecular alterations and a sequence of initiation, promotion, conversion and progression was recognized (Ito *et al.*, 1995). High-risk HPV (HPV16 and 18) infection contributes to carcinogenesis of cervical cancer and tumour progression predominantly through the actions of two viral oncogenes, E6 and E7. These oncogenes are consistently expressed in cervical cell lines and in human cancers (McMurray *et al.*, 2001; Munger & Howley, 2004). Both

of these oncogenes interact with and inhibit the activities of critical components of cell cycle regulatory systems (Philips & Vousden, 1999; Milde *et al.*, 2004; McMurray, 2001). As p53 and pRB normally control cellular proliferation, differentiation, and apoptosis, the abrogation of their normal biological activities places such a cell at a risk of malignant progression. In addition, high-risk HPV E6 and E7 expressing cells have a decreased ability to maintain genomic integrity (White *et al.*, 1994; Margaret *et al.*, 2008).

E6 from the high risk HPVs has been shown to inactivate p53, block apoptosis, activate telomerase, disrupt cell adhesion, polarity and epithelial differentiation, alter transcription and G-protein signaling, and reduced immune recognition of HPV infected cells. The pathways that are targeted by E6 in HPV-associated cancers have provided important insight to identify the critical mutations that are commonly found in other tumours (Howie *et al.*, 2009).

The expression of E7 requires integrity of the p53 tumour suppressor pathway, but does not involve increased expression of p53-responsive genes (Eichten *et al.*, 2004). E7 plays a central role in both viral life cycle and carcinogenic transformation. In the HPV life cycle, E7 disrupts the intimate association between cellular differentiation and proliferation in normal epithelium, allowing for viral replication in cells that would no longer be in the dividing population. This function is directly reflected in the transforming activities of E7, including tumour initiation and induction of genomic instability (Helt and Galloway, 2001; Helt *et al.*, 2002; Margaret *et al.*, 2009).

Available evidence indicates that the most effective way to fight cancer has been to prevent cancer from developing in the first place, either by removing pre malignant lesion, or by chemoprevention via food supplements eats (Milchele and Harvey, 2004). The therapies used in the treatment of cervical cancer are radiotherapy and chemotherapy. Radiotherapy works by damaging a cancer cell's DNA, making it unable to multiply. Radiation therapy can damage nearby healthy cells, cancer cells are highly sensitive to radiation and typically die when treated. Healthy cells that are damaged during radiation are resilient and are able to fully recover. Side effects of radiation vary from patient to patient. It all depends on how often treatment is given. The three most commonly experienced side effects are fatigue skin problems and loss of appetite (Bucci *et al.*, 2005).

Chemotherapy is a cancer treatment that utilizes anticancer drugs. Chemotherapy works by detecting cancerous cells and destroying them. Unfortunately, many chemotherapy drugs cannot discern between healthy and cancerous cells, thus causing side effects like hair loss and stomach upse (Fischer *et al.*, 2003; Skeel *et al.*, 2003).

Natural products play a relevant role in cancer therapy today with substantial number of anticancer agents used either in the natural form or derived from natural products from varius sources such as plants, animals and microorganism (Cheryl *et al.*, 2000). There is a long history of medicinal use of plants in Southeast Asian countries, some of which have been proven to be useful as pharmaceuticals (Burkill, 1966). That is why there is a need to look for agents from commontly consumed natural resources such as vegetable.

Research on the natural products for cervical cancer therapy represents an area of great interest in which plants are the most important source. Besides fulfilling the physiological needs, routine or habitual consumption of vegetables may deliver significant benefits to human health. Dietary modification by increasing the consumption of wide variety of fruits and vegetables daily is a practical strategy for consumers to optimize their health and reduce the risk of cancer. Use of dietary supplements, nutraceuticals and functional foods is increasing as industry is responding to consumers demand (Rui, 2004).

Numerous epidemiological studies have demonstrated a lower risk of cancer among individuals whose diet includes a relatively large amount of vegetable, fruits and plant products, all containing different vitamins and macronutrients with ability to prevent carcinogenesis by interfering with detrimental actions of mutagens, carcinogens and tumour promoters (Hanausek *et al.*, 2001).

In the cancer field, a number of important new commercialized drugs have been obtained from natural sources, by structural modification or natural compounds, or by the synthesis of new compounds, designed following a natural compound as a model. The search for improved cytotoxic agents continues to be important in the discovery of modern anticancer drug (Nobili *et al.*, 2009).

The aims of the present study were:

- a. to screen the crude methanol and water extracts from 40 selected vegetables for cytotoxic potentials against cervical cancer-derived cell lines, CaSki (HPV 16-containing cell lines), HeLa (HPV 18-containing cell lines) and normal cell lines MRC5 (human lung fibroblast) using the neutral red cytotoxic assay.
- b. to screen the crude methanol and water extracts from 40 selected vegetables for the ability to suppress or inhibit the expression of HPV 16 E6 oncoprotein in CaSki cell line using the 3-step indirect avidin-biotin immunoperoxidase immunocytochemistry technique.