INVESTIGATION OF CHEMOPREVENTIVE ACTIVITY WITH TWO DIFFERENT VARIETIES OF FREEZE DRIED COCONUT WATER (FDCW) ON CERVICAL CANCER CELL LINE

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By

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DEDICATIONS

To my beloved father and mother,

Ramlee Bin Mansor *and* ***Saadiah Binti Ismail****,*

My dearest siblings and companions

For making what I am now

"And seek help through patience and prayer, and indeed,

it is difficult except for the humbly submissive [to Allah]"

(Al-Baqarah: 45)

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LIST OF ABBREVIATION

ATCC - American Type Cell Culture

EDTA - Ethylenediamine tetra acetic acid

FDCW - Freeze Dried Coconut Water

CO_2 - Carbon dioxide

DI H_2O - Deionized water

DMEM - Dulbecco's Modified Eagle Medium

DMSO - Dimethyl sulphoxide

DNA - Deoxyribonucleic acid

FBS - Foetal Bovine Serum

PBS - Phosphate Buffered Saline

ROS - Reactive Oxygen Species

HPV - Human papilloma virus

HIV - Human immunodeficiency virus

rpm - Revolution per minute

RT - Room temperature

SEM - Standard error mean

TBEA - Trypan blue exclusion assay

SPSS - Statistical Package for Social Science

LIST OF SYMBOLS

µg/ml	-	Microgram per mililitre
µl	-	Microlitre
mg/ml	-	Milligram per mililitre
g	-	gram
kg	-	kilogram
cm	-	centimetre
mm	-	milimetre
ml	-	mililitre
µm	-	micrometre
µl	-	microlitre
N	-	Normality
ºC	-	Degree celcius
%	-	percentage

PENYIASATAN AKTIVITI PENCHEGAHANKEMO DENGAN MENGGUNAKAN DUA VARIETI AIR KELAPA YANG DISEJUK-KERINGKAN KE ATAS TITISAN SEL KANSER SERVIK

ABSTRAK

Air kelapa dianggap sebagai penawar semulajadi untuk manusia. Bukan sahaja sebagai minuman yang menyegarkan untuk menghilangkan dahaga, air kelapa digunakan sejak dari bayi ke alam dewasa sebagai tonik kesihatan terutamanya oleh orang-orang di kawasan tropika. Dengan peningkatan penyakit kanser, terapi antikanser baru seperti pendekatan pencegahan-kemo diperlukan dengan kadar segera. Dalam kajian ini, didapati bahawa air kelapa menunjukkan aktiviti perencatan pertumbuhan terhadap sel HeLa dengan nilai IC_{50} 100 μg/ml bagi kedua-dua varieti air kelapa MATAG dan air kelapa Rendah Aromatik yang telah disejuk-keringkan. Selepas 72 jam rawatan, pemerhatian menggunakan mikroskop menunjukkan perubahan morfologi apoptotik dalam sel HeLa yang dirawat menggunakan air kelapa MATAG yang telah disejuk-keringkan. Sementara itu, ciri-ciri apoptosis dan autofagi dapat diperhatikan dalam sel HeLa yang dirawat meggunakan air kelapa Rendah Aromatik yang telah disejuk-keringkan. Sebaliknya, pemerhatian di bawah mikroskop pendarfluor menunjukkan intens kromatin yang tinggi dan morfologi apoptotic terdapat di dalam kedua-dua jenis rawatan terhadap sel HeLa yang telah diwarnakan dengan pewarna Hoechst. Untuk menilai aktiviti anti-proliferatif dalam tempoh rawatan yang berpanjangan, sel HeLa dirawat dengan air kelapa MATAG dan air Kelapa Rendah Aromatik yang telah disejuk-keringkan selama lapan hari tempoh pembiakan. Hasil kajian menunjukkan bahawa air kelapa MATAG yang telah disejuk-keringkan mampu untuk terus merencat percambahan sel HeLa sehingga hari terakhir rawatan. Sebaliknya, kesan perencatan air kelapa Rendah Aromatik yang telah disejuk-keringkan adalah tidak stabil dalam tempoh masa yang lama, oleh itu kesan perencatan berkurangkan. Dengan itu, hasil kajian menunjukkan bahawa air kelapa MATAG yang telah disejuk-keringkan mempunyai kesan anti-proliferatif lebih baik berbanding dengan air kelapa Rendah Aromatik yang telah disejuk-keringkan. Kesimpulannya, kedua-dua jenis air kelapa yang telah disejuk-keringkan menunjukkan aktiviti penchegahankemo yang positif seterusnya dianggap sebagai terapi antikanser yang berpotensi.

INVESTIGATION OF CHEMOPREVENTIVE ACTIVITY WITH TWO DIFFERENT VARIETIES OF FREEZE DRIED COCONUT WATER (FDCW) ON CERVICAL CANCER CELL LINE

ABSTRACT

Coconut water is considered as nature's elixir to mankind. Not only as a refreshing beverage to quench the thirst, coconut water is consumed starting from infancy to adulthood as a health tonic especially by people of the tropics. With increasing trend of cancer prevalence, novel anticancer therapies such as chemoprevention approach urgently needed. In this study, it was found that coconut water exert its anti-proliferative activity against HeLa cells with IC_{50} value of 100 µg/ml in both MATAG FDCW and Aromatic Dwarf (AD) FDCW varieties. After 72 hours of treatment, observation under inverted microscope showed typical apoptotic morphological alteration in HeLa cells exposed with MATAG FDCW. Meanwhile, features of apoptosis and autophagy observed in HeLa cells treated with AD FDCW. Observation under fluorescence microscope showed chromatin condensed and apoptotic bodies in both treatments against stained HeLa cells with Hoechst dye. To evaluate the anti-proliferative activity with prolong treatment exposure, treated HeLa cells with respective treatments were subjected to eight days of incubation period. Results showed that MATAG FDCW was able to continuously suppressed HeLa cells proliferation until final day of treatment. However, AD FDCW effect was instable with prolonged time hence, the suppression effect was reduced. Thus, the results suggested that MATAG FDCW exerts better anti-proliferative effect compared to AD FDCW. In conclusion, both FDCW varieties demonstrated positive chemopreventive activity thence considered as potential novel anticancer therapies.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Cancer is claimed to be one of the most leading causes of death worldwide. Out of three people, one person is diagnosed with cancer (Rang HP, 2007). In 2008 it was reported that about 12.7 million people were diagnosed with cancer and 7.6 million people lost their lives to cancer annually. By 2030, cancer is predicted to ascend over 20 million (Jemal *et al.*, 2011) ; (Rang HP, 2007). According to National Cancer Registry Report, it was reported in 2007 that the common cause of death in hospitals after heart diseases and septicemia was cancer. New cancer morbidity was diagnosed with the total of 18, 219 cases in 2007 whereby, it comprises of 44.6% males and 55.4% females (Omar and Tamin, 2011).

Cervical cancer is primarily a preventable disease, yet it remains the third most common cancer in women worldwide with estimated new cases up to 529, 800 (Jemal *et al.*, 2011). Likewise, in Malaysia cervical cancer ranked in the third position which accounts 847 cases of total most frequent cancers among females (Omar and Tamin, 2011). Nevertheless, cervical cancer morbidity and mortality have been reduced through screening and treatment in earlier stages (Teoh *et al.*, 2015). Even so, modern conventional therapies are ineffective since the overall cancer burden is still high. Hence, this has created an imperative need for novel intervention (Neergheen *et al.*, 2010).

With this concern, numerous researches have been done to develop new therapeutic strategies in combating this terrifying disease. Chemoprevention is among the active area of investigation with enormous potential. As was first introduced by Sporn in 1976, chemoprevention is an attempt to prevent, reverse, or suppress carcinogenic progression by the use of natural, biological, chemical, or synthetic agents. This approach tackles carcinogenesis from the initial phases until the progression states (Sporn, 1976); (Sporn and Suh, 2000); (Tsao *et al.*, 2004).

Natural phytochemicals or dietary supplements have gained an upsurge of interest in the field of cancer prevention and therapy. Traditional herbs, folk medicine, or functional food have been long used by Asians since thousand years ago to prevent or treat ailments in the prior of the introduction of Western Medicine. At present, numbers of scientific data have been collected which emphasized the interrelationship of lifestyle-related diseases like cancer, heart disease, diabetes, etc. It is believed that right diet modification in daily food intake can prevent such chronic diseases (Iqbal Ahmad, 2006).

Through research, dietary phytochemicals which can be found in vegetables, spices, fruits, and beverages have exhibited potential anticancer properties. Their anti-cancer activities can be learned through multiple pathways and mechanisms. Dietary phytochemicals play multiple roles in the normal biological processes and also involved in the regulation of pathological progressions. Moreover, dietary phytochemicals generally have low toxicity level, hence making these bioactive natural compounds as an essential cancer chemopreventatives agents (Shankar *et al.*, 2013); (Shukla *et al.*, 2014).

Malaysia is a tropical country endowed with vast natural resources. The favorable climate and soil conditions present this country with rich biological diversity hence, renowned as one of the world's twelve megadiversity countries (Hezri and Nordin Hasan, 2006). Nevertheless, the scientific data regarding our nation's natural resources are lacking and yet to be investigated (Hezri and Nordin Hasan, 2006). For that, this study is carried out as an effort to document the scientific evidence about one of the nation natural resources, specifically coconut water. The purpose of this study is to investigate the cancer chemoprevention activity with two different varieties of coconut water on cervical cancer cell line.

Coconut water has been used especially in the tropical countries not only as a refreshing beverage but it is also considered as health tonic. The Hawaiians refer coconut water as "the dew from the heavens" due to its enormous benefits. The folks believed that coconut water is useful in the treatments for poisoning, dehydration, heatstroke, boils, fatigue, urinary tract infections, kidney stones, constipation, digestive disturbances, diarrhea, malnutrition, osteoporosis, and sterility (Prades *et al.*, 2012); (Fife, 2008). With vast benefits of coconut water, it is believed that this health beverage will exhibit positive chemoprevention activity on cancer cells. In this study, the chemoprevention activity of coconut water from two different varieties, MATAG and Aromatic Dwarf, were determined by their ability to suppress or inhibit the growth of cervical cancer cells.

1.2 HYPOTHESIS

The hypothesis of this study is that the different varieties of freeze dried coconut water will exhibit different types of chemoprevention activity with regards of inhibitory concentration dose and cell proliferation.

1.3 OBJECTIVES

1. To determine the inhibitory concentration with two different varieties of freeze dried coconut water on cervical cancer cell line.
2. To observe any morphological changes after treatment with two different varieties of freeze dried coconut water on cervical cancer cell line.
3. To identify the anti-proliferative effects with two different varieties of freeze dried coconut water on cervical cancer cell line.

The experimental design flowchart of this study is shown in APPENDIX A.

CHAPTER 2

LITERATURE REVIEW

2.1 Cancer

Generally, cancer is linked to death since it is incurable and even the etiology is not fully understood. Thence, cancer has become a terrifying illness and it is in the center of attention in medical field (King and Robins, 2006). Regardless the declaration of "war on cancer" by US government in 1971, the morbidity of cancer cases in the recent years have decreased not more than 1% and the mortality only reduced not more than 2% (Barbara *et al.*, 2014) ; (Siegel *et al.*, 2012). To add, the prevalence of cancer burden and incidence was reported as the highest in economically developing countries, which include Malaysia. Moreover, the survival also tend to be poorer in developing countries (Bray *et al.*, 2012).

The term cancer, malignant or benign neoplasm marked by uncontrolled multiplication of abnormal cells. The pathogenesis of cancer manifest to varying levels and classified by four characteristics that distinguish them from normal cells. Features of cancer cells include uncontrolled proliferation, dedifferentiation and loss of function, invasiveness, and aptitude to spread or metastasize to other parts of the body (Rang HP, 2007). Due to mutation, a normal cell turns into a cancerous cell either by inherited or acquired. It is truly a complex process of progressing genetic modification in human somatic cells and often with no fixed molecular basis. Although the genetic predisposition factors or infectious pathogens can markedly increase the risk of cancer, neither of those alone is necessary and sufficient to cause malignancy (Barbara *et al.*, 2014).

Carcinogenesis defined as the process whereby benign epithelial cells acquire attributes of neoplastic malignancy. Deregulation of sound orchestrated process cause the normal cells to turn into malignant cells. The pathogenesis of cancer metastasis comprises of selective, sequential, and interlinked steps. Aftermath of the metastatic process depends on both intrinsic properties of the tumor cells and their interaction with host factors (Langley and Fidler, 2007). Figure 2.1 shows the pathogenesis of cancer metastasis.

Cancer carcinogenesis is a multistage process that includes the initiation phase, promotion phase, and progression phase (Khambete and Kumar, 2014). The initiation phase is due to carcinogenic insult, in which it involves with environmental exposure such as toxic chemicals or radiations. Following the insult, cells will undergo promotion phase whereby the initiated cells turn to non-malignant tumor cells (adenoma) (Kasdagly *et al.*, 2014). Later on in the progression phase, cells transformed to neoplastic malignant cells which are irreversible and they acquire the ability to invade and metastasis. Therefore, cancer is not merely to be removed or killed, but it is a course to be prevented, managed and controlled (Barbara *et al.*, 2014).

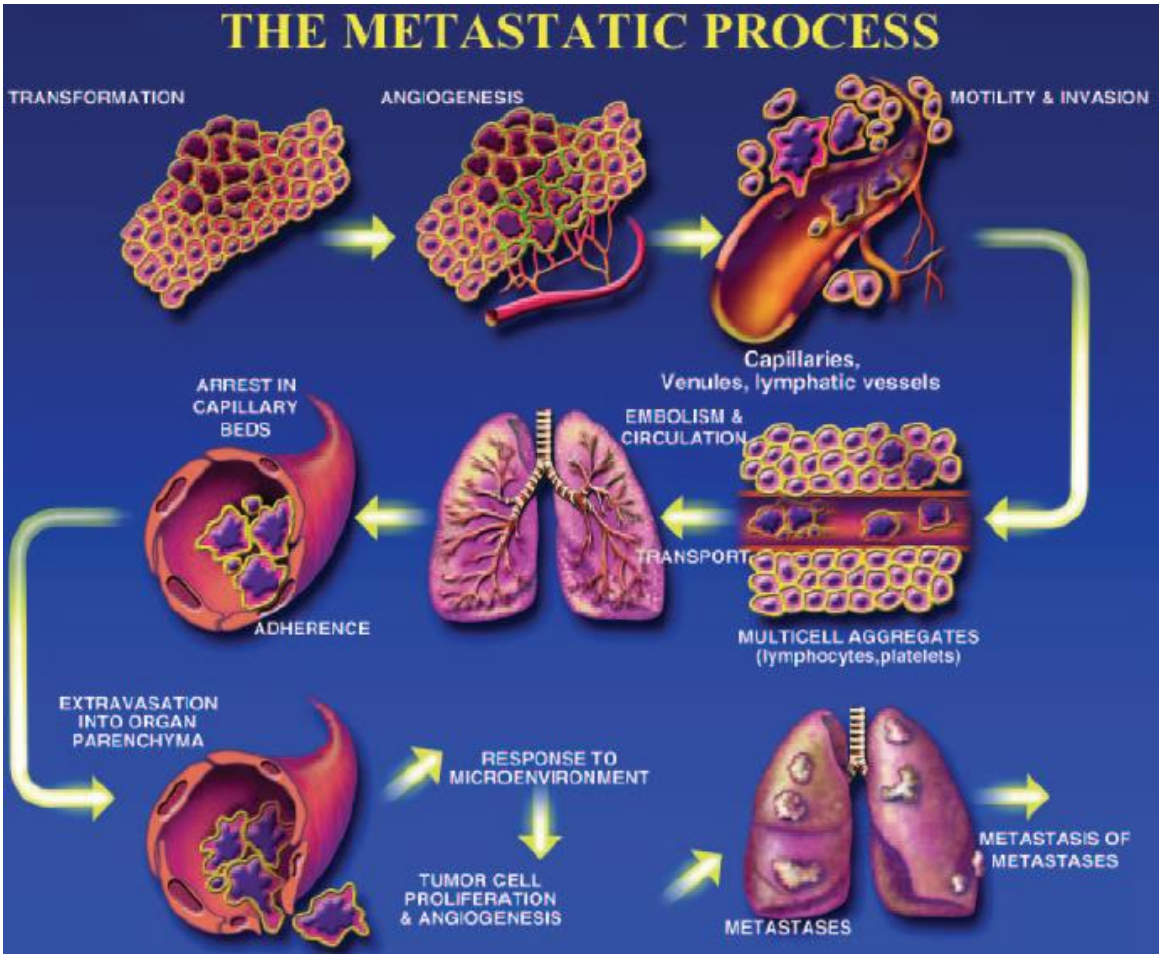


Figure 2.1: Pathogenesis of Cancer Metastasis.

(Adapted from Langley and Fidler, 2007)

2.1.1 Hallmarks of cancer

Cancer is widely specified as a group of diseases with common hallmarks of uncontrolled growth and life-threatening (King and Robins, 2006). To understand the diversity of neoplastic disease, Hanahan and Weinberg (2000) had proposed six hallmarks of cancer establishing an organizing principle that provides a coherent framework. These hallmarks concept discussed on the notion that a normal cell change progressively to a neoplastic state. It also emphasized that the normal cells acquire the attributes that facilitate them to become tumorigenic and ultimately malignant (Hanahan and Weinberg, 2011). Figure 2.2 depicts the proposed hallmarks.

The first hallmark, which is the vital trait of cancer capabilities, is the ability to sustain the proliferative signaling. This growth-promoting signal continuously being released and regulates progression through cell cycle as well as cell growth hence, becomes immortalized (Hanahan and Weinberg, 2011). The second hallmark of cancer cells is that they were able to evade growth suppressors by circumvent tumor suppressor genes actions. For instance, prototypical tumor suppressors that encode TP53 and RB (retinoblastoma-associated) proteins govern the decision of cells whether to proceed or not through its growth-and-division cycle. The growth suppressors will determine if a cell should continue to proliferate or undergo senescence and apoptotic programs (Burkhart and Sage, 2008).

Cancer cells usually have defects in RB or TP53 pathways thus, lost the critical gatekeeper of cell-cycle and allowing persistent cell proliferation. Evading cell death is the third cancer hallmark capability. Cancerous cells develop a variety of strategies to bypass apoptosis to succeed in malignancy states and become resistance to therapy. Malignant cells

could induce and increase the expression of antiapoptotic regulators such as Bcl-2 and Bcl-x_L or survival signals like Igf1/2. Commonly, cancer cells lost the function of TP53 therefore eliminates apoptosis-inducing circuits (Adams and Cory, 2007).

The next hallmark is enabling replicative immortality of cancer that results in generation of macroscopic tumor. Previous researches had demonstrated that the immortalized variant cells were capable of maintaining the length of telomeric DNA that sufficient to evade triggering of apoptosis or senescence pathways (Blasco, 2005); (Hanahan and Weinberg, 2011). Another integral hallmark of cancer is inducing angiogenesis. In order to remain alive, tumor cells also need sustenance such as oxygen and nutrients to nourish themselves. During tumor progression, "angiogenic switch" is constantly activated thence the development of vasculature and angiogenesis persist facilitating neoplastic growth.

Another hallmark capability is activating invasion and metastasize. Local invasion and distant metastasis are the highest pathological states of malignancy. Alterations in shape and interaction to extracellular matrix and cell-to-cell adhesion molecules (down-regulation of E-cadherin) explained this condition (Berx and Van Roy, 2009). Hanahan and Weinberg (2011) later came out with new emerging hallmarks and enabling characteristics as addition to the previous hallmarks. The two enabling characteristics are genome instability and mutation and tumor-promoting inflammation whilst the two emerging hallmarks are reprogramming energy metabolism and evading immune destruction. Figure 2.3 illustrates this emerging hallmarks and enabling characteristics. Understanding the hallmarks of cancer is important in order to target each of the cancer hallmarks capabalities, enabling characteristics and emerging hallmarks for therapeutics or chemoprevention interventions.

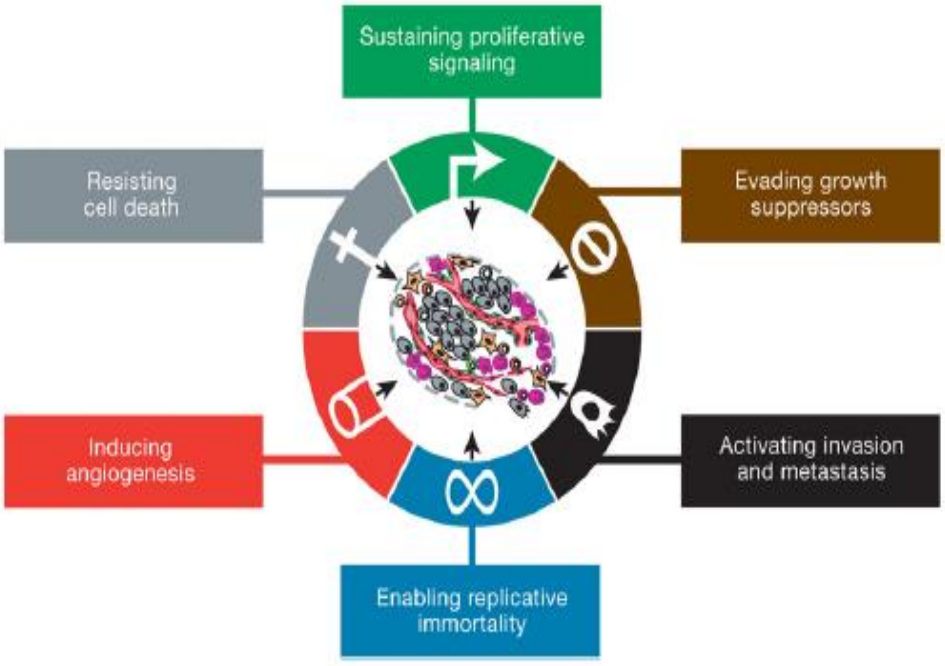


Figure 2.2: Common Hallmarks of Uncontrolled Growth.

(Adapted from Hanahan and Weinberg, 2011).

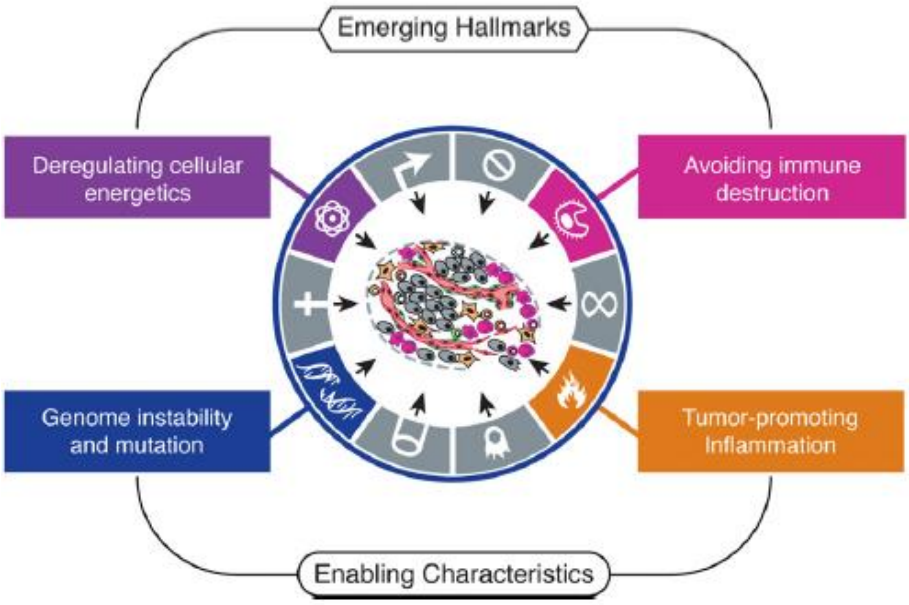


Figure 2.3: Emerging Hallmarks and Enabling Characteristics.

(Adapted from Hanahan and Weinberg, 2011).

2.2 Cervical Cancer

2.2.1 Anatomy of cervix

The uterus also known as womb serves as the pathway for sperm to be deposited, site of fertilized ovum implantation, development of fetus during pregnancy and labor. Uterus also the source of menstrual flow during reproductive cycle. Anatomical subdivision of the uterus classified into fundus, body, and cervix, meanwhile between the body and cervix there is isthmus. The cervical canal open into the uterine cavity at the internal os and into the vagina at the external os (Tortora and Derrickson, 2009). The anatomy of cervix is shown in Figure 2.4.

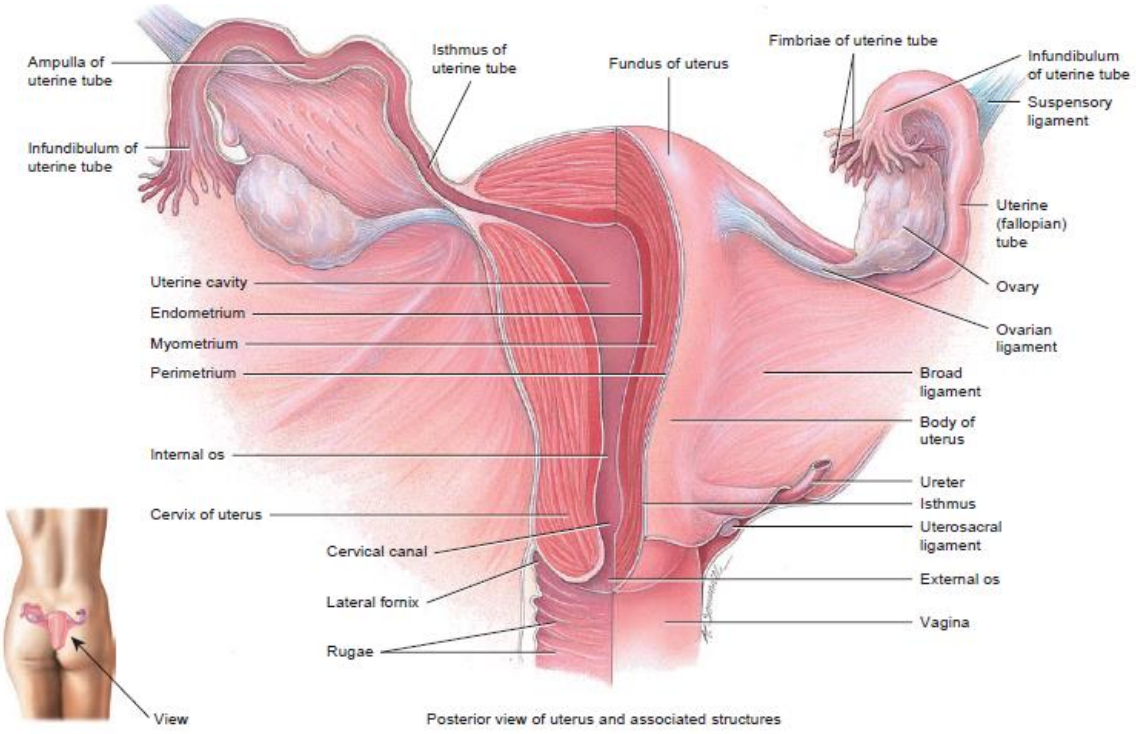


Figure 2.4: Overview image of the cervix.

Adapted from (Tortora and Derrickson, 2009).

2.2.2 Types of cervical cancer.

There are two main types of cervical cancer namely squamous cell carcinoma and adenocarcinoma. Most common cervical cancer are adenocarcinomas, which account up to 9 out of 10 occurance. Cervical adenocarcinoma advances from the mucus-producing gland cells of the endocervix whereby it gradually develops pre-cancerous modifications that turn into cancer. Several terms to describe these pre-cancerous alterations include squamous intraepithelial lesion (SIL), cervical intraepithelial neoplasia (CIN), and dysplasia. Early detection by Pap test can treat and prevent these changes and stop cancer from developing (American Cancer Society, 2014). Location of cancerous tissue in the cervix is shown in Figure 2.5.

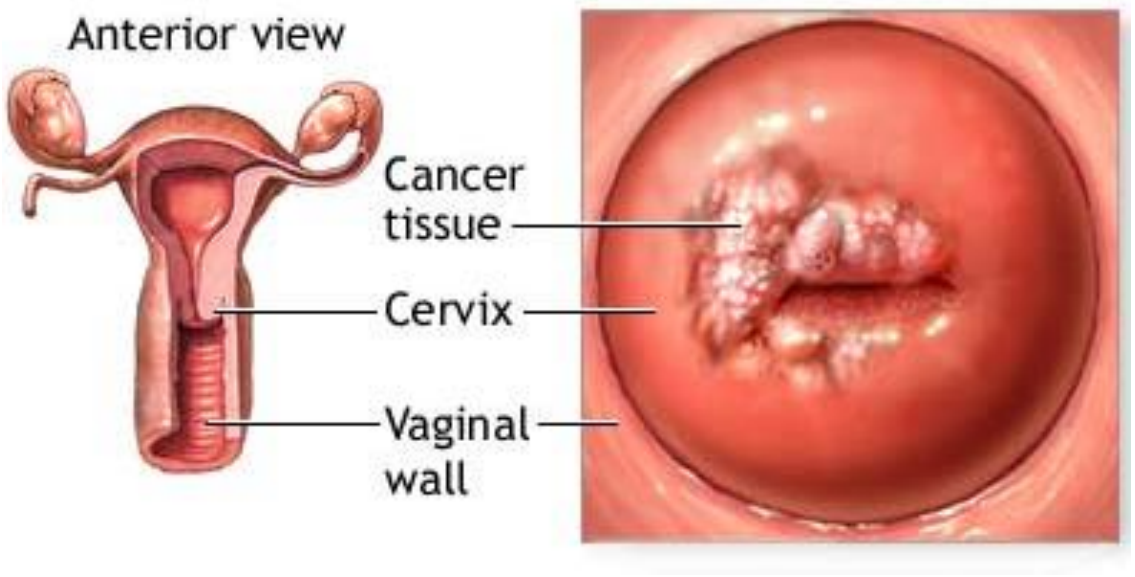


Figure 2.5: Cancerous tissue in the cervix

(Adapted from A.D.A.M. Inc., 2013).

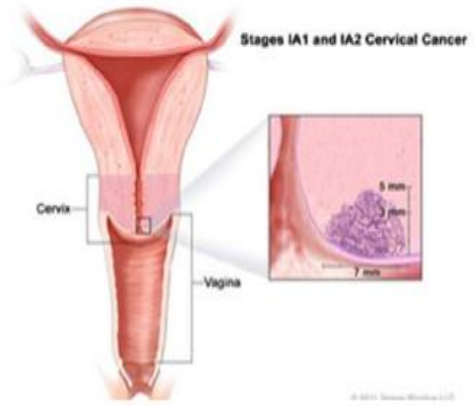
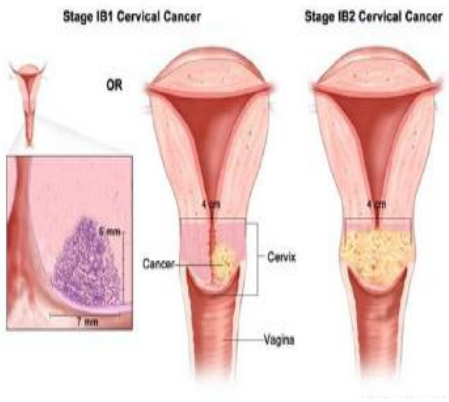
2.2.3 Staging and symptoms of cervical cancer.

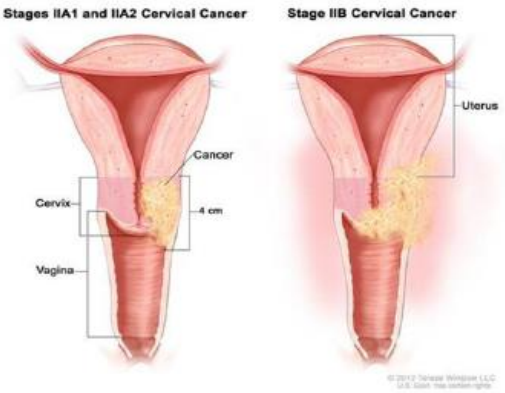
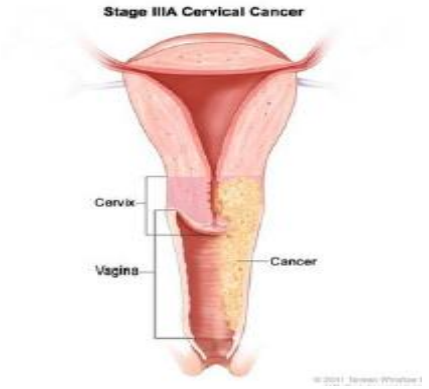
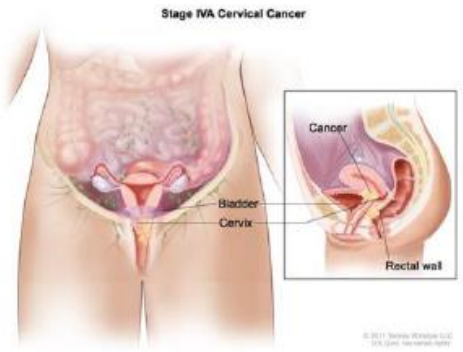
Cancer of cervix is a disease wherein cancerous cells develop in the tissues of female uterine cervix. Initiation of cervical cancer normally arises in cells on the surface and over time, the cancerous cells can invade deeper into the cervix and nearby tissues (Sushma *et al.*, 2014).

The extent of the cancer often expressed in term of its stage. The stage of classification is essentially based on clinical examination by means of anatomical extent of the disease. It provides information on the size of the tumor, how deep it has grown in the tissues and surrounding cervix and how far it has metastasized. In early stage, or widely referred as Stage I is classified when the lesion appear to be confined to the organ of origin. At Stage II, the tumor has extended locally beyond the site of origin to implicate with adjacent structures or organs. Whereas at Stage III, there is more extensive involvement can be noticed. And later at Stage IV, notable metastatic spread can be seen. These are the basic stages which then further classified into substages, wherein often a reflection of specific prognostic factors within a given stage (Benedet *et al.*, 2000). The clinical stages of cervical cancer are shown in Table 2.1.

The clinical manifestation of early cervical cancer is generally asymptomatic whereby the lesion is not visible but detectable through routine cytology screening. However, patient with visible lesion usually experience abnormal vaginal bleeding between periods, menstrual flows that last longer and heavier, increase vaginal discharge, pelvic pain and so forth (Levenback, 2006); (Sushma *et al.*, 2014).

Table 2.1: The stages of invasive cervical cancer are as following: (Adapted and modified from (Levenback, 2006); (Sushma *et al.*, 2014).

Stage 0: Carcinoma in situ. **Stage I**: Cervical carcinoma confined to uterus. **Stage IA1**: Measured stromal invasion in horizontal spread not more than 3 mm in depth and not wider than 7 mm in diameter. **Stage IA2:** Stromal invasion measured greater than 3 mm but not more than 5 mm in depth and spread not more than 7 mm in diameter.	
Stage IB: Lesion clearly visible confined to the cervix or microscopic lesion greater than Stage IA. **Stage IB1**: Size of visible lesion not greater than 4 cm **Stage IB2**: Clinical lesion greater than 4 cm in dimension	

Stage II: Cervical carcinoma invades beyond uterus but not to pelvic wall or to the lower third of vagina **Stage IIA**: No obvious parametrial invasion **Stage IIB**: Obvious parametrial invasion but not into the pelvic sidewall.	
Stage III: Cervical carcinoma extends to the pelvic wall and/or involves lower third of vagina or causes hydronephrosis or nonfunctioning kidney. **Stage IIIA**: Tumor extends to lower third of the vagina, but not involves the pelvic wall. **Stage IIIB**: Extension of tumor into pelvic wall or causes hydronephrosis or nonfunctioning kidney	
Stage IVA: Tumor invasion up to mucosa of bladder or rectum and/or extends beyond pelvis **Stage IVB**: Distant metastasis	

2.2.4 Risk factors of cervical cancer

Statistically, cervical cancer has been reported to be more prevalent among rural women than urban women in developing and developed countries. This situation can be linked with factors such as low levels of education, unemployment, living in poverty and barriers to preventive screening (Wong, 2011).

Over centuries, it has been well understood that cervical cancer is concomitant of sexual activity. The necessary event for the development of cervical cancer is the persistence of Human Papilloma Virus (HPV) in the cervix (Moscicki *et al.*, 2012). It is widely accepted that HPV is transmitted through sexual contact. Hence, it clarifies the epidemiological association concerning cervical cancer incidence and number of sexual partners (Waller *et al.*, 2004).

HPV belongs to the *Papillomaviridae* family and over 200 genotypes have been identified. Examples of high-risk HPV serotypes include 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82 and should be considered as carcinogenic. The probable high-risk types comprise of types 26, 53, and 66 while, HPV classified as low-risk types are 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and CP6108 (Munoz *et al.*, 2003). Papillomaviruses are simple, non-enveloped and have double stranded deoxy-ribonucleic acid (DNA). The low-risk HPV serotypes can cause benign lesion whereas the high-risk HPV serotypes can result in malignant lesion. This virus is transmitted via direct contact of mucosa membrane or skin and infects the epithelial cells. It requires the presence of micro abrasions or micro trauma in the stratified squamous epithelium layer to facilitates penetration into the basal cell layer of the cervixdits tropic destination (Haugsdal and Ryan, 2014). The virus will then reproduce

within the host cells and after the host cells die, new virus particles will be released and start to infect other surrounding cells.

To add, series of co-factors also involved in the development of cervical cancer. For instance, high parity, long-term use of oral contraceptives, immunological factors, smoking and other sexually transmitted infections (STIs) have been established as risk cofactors for cervical cancer. Some other intervening factors are human immunodeficiency virus, *Chlamydia trachomatis,* herpes simplex simplex virus type 2, and poor dietary (Bosch and De Sanjosé, 2002); (Waller *et al.*, 2004).

2.2.5 Screening tools and diagnostic test

HPV is the only gynecologic cancer that can be prevented by regular screening. Screening programs have effectively reduced the burden of cervical cancer in developed countries. However, screening and prevention programs face major hurdle like cost constraints, loss to follow-up, poor participation, and concerns about sustainability hence becomes a barrier in developing countries. Thus, there are efforts to improve the test sensitivity and specificity yet affordable (Belinson *et al.*, 2014).

Pap smear cervical cytology is still considered as primary method for early diagnosis of precursor lesions of cervical carcinoma. It is not a diagnostic but a screening test and better known as Pap test. It is well understood that no single screening method exist has highly specific, highly sensitive, practical and affordable. Hence, other methods have been employed to complement the findings of the conventional Pap test such as electron microscopy, immunohistochemistry, molecular biology, biomarkers, HPV DNA, polymerase

chain reaction (PCR) or by the hybrid capture system (Michalas, 2000); (Dasari *et al.*, 2015). Specimens are obtained by scarping cells at the surface of cervix and vagina to be examined.

2.2.6 Vaccination.

Disease prevention is the principle strategy in dealing with any disease incidence. Well-established etiologic connection between cervical cancer and HPV infection imposed the need of vaccination (Gattoc *et al.*, 2013). Through vaccination, the burden of cervical cancer has significantly reduced in developed countries. It is the primary prevention with aim to circumvent a disease in its entirety (Haugsdal and Ryan, 2014)**.** The ideal vaccination target would be the young adolescents who have the highest risk of HPV infection. It is believed that the earlier a person start the sexual contact, the more likely for them to have a persistent HPV infection (Al-Naggar *et al.*, 2010).

The Food and Drug Administration (FDA) has approved numbers of vaccines in the markets. The first vaccine introduced by Merck and approved in 2006 is Gardasil vaccine. It is a non-live quadrivalent vaccine based on the L1 capsid protein that needs to be administered in 3 injections series: on day 0, at 1-2 months, and at 6 months. The second vaccine, Cervarix, introduced by GlaxoSmithKline was approved in 2009. This vaccine is bivalent which also exploits L1 viral capsid protein as the basis of its efficacy. Cervarix also gives protection against anal, vaginal, genital warts, and vulvar cancers. The administration is similar to Gardasil schedule. The safety and efficacy evaluation of both HPV vaccines established 100% sero-conversion and neither demonstrated any significant or serious adverse effects (Gattoc *et al.*, 2013); (Haugsdal and Ryan, 2014).

Nevertheless, despite well-established guidelines and recommendations, the developing countries like Malaysia and other poor countries faced the greatest barriers to implement vaccination program. Due to cost constraint, deprived access to effective screening and treatment programs, dissemination of vaccines and social cultural norms make vaccination program a challenging effort (Wong, 2011).

2.2.7 Cervical cancer treatment and its side effects.

To date, the framework for cancer treatment comprises of surgical removal of abnormal tissue mass, pro-apoptotic cancer therapeutics such as chemotherapy, radiotherapy, immunotherapy, hormone therapy or combination depending on stage of disease and histologic type (DeSantis *et al.*, 2014). Patients with early-stage disease (Stage IA1), their primary treatment would be surgery or radiation therapy. Surgery of extrafascial hysterectomy, modified radical trachelectomy or hysterectomy with pelvic node dissection is recommended (Sushma *et al.*, 2014). While patients with advanced metastatic and recurrent disease are treated with surgery followed by radiation therapy (brachytherapy) or chemotherapy (cisplatin, bevacizumab, docetaxel, 5-FU,) or combination (DeSantis *et al.*, 2014); (Sushma *et al.*, 2014).

Although conventional cancer therapeutics have prolonged the survival, the problem is that they often off-target in discriminating normal cells and cancerous cells. Consequently, patients faced substantial risk of adverse effects. To exemplify, in radiation therapy “hot spots” were observed in normal tissue and sometimes high volumes of radiation in rectum and bladder are unavoidable. Besides, patients experiencing acute and chronic toxicity in gastro-intestinal system and rectal-lymphatic drainage are common. (Du *et al.*, 2012);

(Barillot *et al.*, 2014). Other side effects include infertility in case of hysterectomy and menopause symptoms in case of bilateral oophorectomy surgery Moreover, cancer cells are now resistant towards chemotherapy thus, makes the conventional therapy ineffective (DeSantis *et al.*, 2014).

2.3 Cancer chemoprevention.

The persistent magnitude of cancer morbidity, the increasing trend in conventional therapy failure, the recurrence problem after primary cure, and the deterioration of patient's quality of life indicate that new approaches are critically needed (Kundu *et al.*, 2014). In response, there is a rising number in research regarding alternative anticancer therapies and strategies in combating this chronic disease. Among the active area of investigation is chemoprevention.

As was first coined by Sporn in 1976, cancer chemoprevention denotes as pharmacological intervention that utilizes natural, synthetic, or biologic chemical agents to prevent, suppress, or reverse carcinogenic advancement (Sporn, 1976); (Sporn and Suh, 2000). This approach addresses cancer disease as primary, secondary, and tertiary cancer chemoprevention. The primary chemoprevention tackles cancer initiation by reducing the risk of cancer development. Conversely, secondary chemoprevention aims to avert malignancy through eradication or induction of stasis in premalignant lesions. Ultimately, tertiary chemoprevention purports to inhibit the recurrence of cancer after a successful primary cure (Russo, 2007); (Ting *et al.*, 2014). Hence, it is believed that chemoprevention agents are not only capable of preventing cancer but are also able to cure it.

Numerous studies have identified numbers of edible plants possessing potential chemopreventive compounds widely known as phytochemicals. These compounds are classified into two categories: cancer-blocking and cancer-suppressing agents. Cancer-blocking agents prevent cancer initiation by blocking carcinogens from hitting their targets. The mechanisms involved are modifying carcinogen uptake and metabolism, enhancing carcinogen detoxification, scavenging reactive oxygen species (ROS) and other oxidative species and finally enhancing DNA repair. Whereas, cancer-suppressing agents work by inhibiting cancer promotion and progression. These agents interfere with cell signal transduction, transcriptional regulation, cycle regulation and induce apoptosis of pre-neoplastic cells (Russo, 2007). Understanding the mechanisms of cancer chemoprevention is essential for safe application and also permits further expansion of novel treatment regimens for cancer patients (Raffoul *et al.*, 2012).

Dietary chemoprevention is a potential alternative or additional regimen for cancer patients plus it is well-tolerated. Rich phytochemicals consumption in dietary or herbal medicines is a convenient mode of administration of chemopreventive phytochemicals and moreover it is inexpensive (Gullett *et al.*, 2010); (Ting *et al.*, 2014). Examples of chemopreventive agents are curcumin in turmeric, genistein in soy, resveratrol in red grape and many more (Shankar *et al.*, 2013). However, in this study chemopreventive agent of coconut water (*Cocos nucifera*) was investigated.

2.4 Coconut.

2.4.1 Botanical description.

Coconut tree belongs to the family Palmae (*Arecaceae*), subfamily of *Cocoideae*, genus *Cocos L*. and species *Cocos nucifera L*. (USDA, 2015). Botanically, coconut is categorized as seed, while people of the tropics consider it as a fruit. The coconut palms are primarily classified into two distinct groups that are the tall varieties and the dwarf varieties. The tall varieties grow slowly and bear fruits up to 6–10 years after plantation. They are hardy type and live up to 80–120 years of ripe age. The coconut fruit takes 11 to 12 months to reach maturity. On the other hand, the dwarf varieties are fast growing and bear fruits earlier, at age of 4–5 after plantation. They are less hardy compare to tall and require favorable soil type and climate conditions to facilitate better yield (DebMandal and Mandal, 2011).

Coconut palm usually can be found in the tropic lands. The geographical distribution of coconut spread to the region spanning Southeast Asia to the Pacific coast of America and across coastal S. Asia, W. Africa, the New World Atlantic, and the Caribbean (Gunn *et al.*, 2011). In Malaysia, there are 13 varieties of coconut can be found which comprises of:

1) Malayan Tall
2) Malayan Red Dwarf (MRD)
3) Malayan Yellow Dwarf (MYD)
4) Malayan Green Dwarf (MGD)
5) West African Tall
6) Tagnanan Tall
7) Rennel Tall
8) Aromatic Dwarf