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Evaluation of Phyllanthus, for Its Anti-Cancer Properties

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1. Introduction

1.1 Cancer

Cancer is a name given to a group of diseases that arise from a single cell when it starts to grow abnormally in an uncontrollable manner to form a group of undifferentiated cells, known as tumour. Tumour can be classified into two categories; benign and malignant. Not all benign tumours are cancerous but all malignant tumours are (Hanahan & Weinberg, 2000). The main difference between these tumours is benign tumour lack the metastatic ability, grows locally and is less harmful. However, some benign tumours can transform into malignant tumours that possess the metastatic ability to invade and spread to other parts of the body via the blood or lymphatic circulation and form secondary tumour and eventually lead to death (Vincent & Gatenby, 2008).

1.2 Development of cancer (carcinogenesis)

Cancer develops through a multistep process known as carcinogenesis (Fig 1), which includes initiation, promotion and progression (Pitot, 2006). An initiation stage is a permanent and irreversible event, which involves one or more cellular changes arising upon exposure to carcinogens, which leads to alteration in DNA and may result in a mutated cell to divide rapidly (hyperplasia). These transformed (initiated) cells can remain harmless, unless exposed to a stimulator, which enhances the tumour to grow into a larger mass. This is a reversible process and is known as the promotion stage. The progression stage is an irreversible conversion of a benign tumour to become a malignant tumour. This carcinogenesis process usually takes 10 years or more to develop and usually depends on the internal (life style) and external (environmental) factors of the patient (Pitot, 2002).

1.3 Hallmarks of cancer

A transformed cell has to acquire six hallmarks in order to be developed into cancer (Fig 2) (Hanahan & Weinberg, 2000). Each of these hallmarks is derived upon changes in the normal cell's physiology and interacts with each other to promote malignant growth. The conversion from a normal cell to become a transformed cell usually starts from mutations in DNA which cause the cells no longer depend on growth signals, thus gaining uncontrolled growth and proliferation. The irresponsiveness or insensitivity to anti-growth signals in

cancer cells, enables them to evade apoptosis. The mutations in cancer cells can also change the normal function of the telomerase, thus give rise to cancer cell a limitless replicative potential (Bree et al. 2002; Kim et al. 1994). Tumour invasion, metastasis and angiogenesis play important roles in allowing the progression of a malignant tumour, by escaping the primary site, invading into blood or lymph circulation, migration to a distant site and finally establishing a secondary tumour.
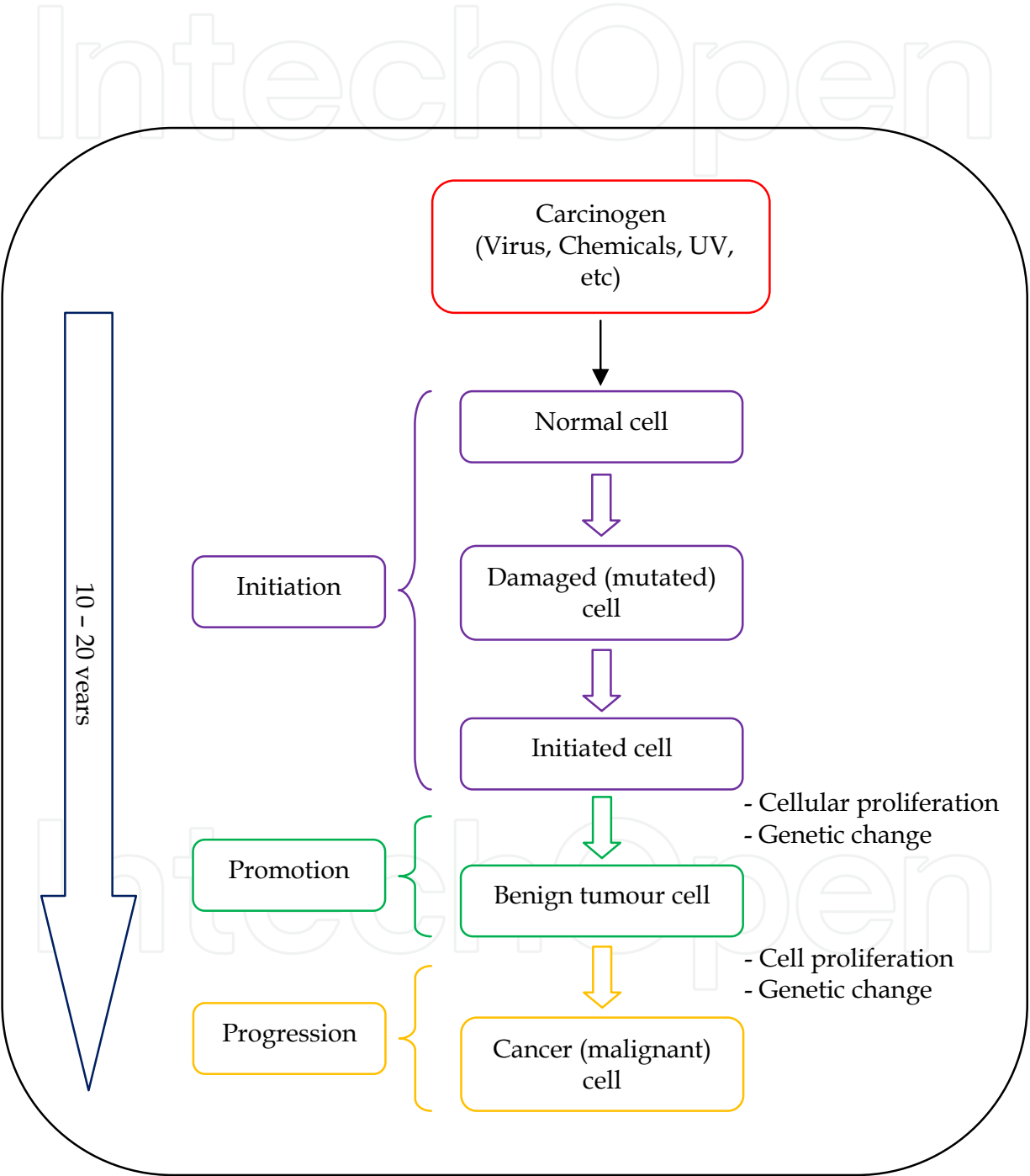

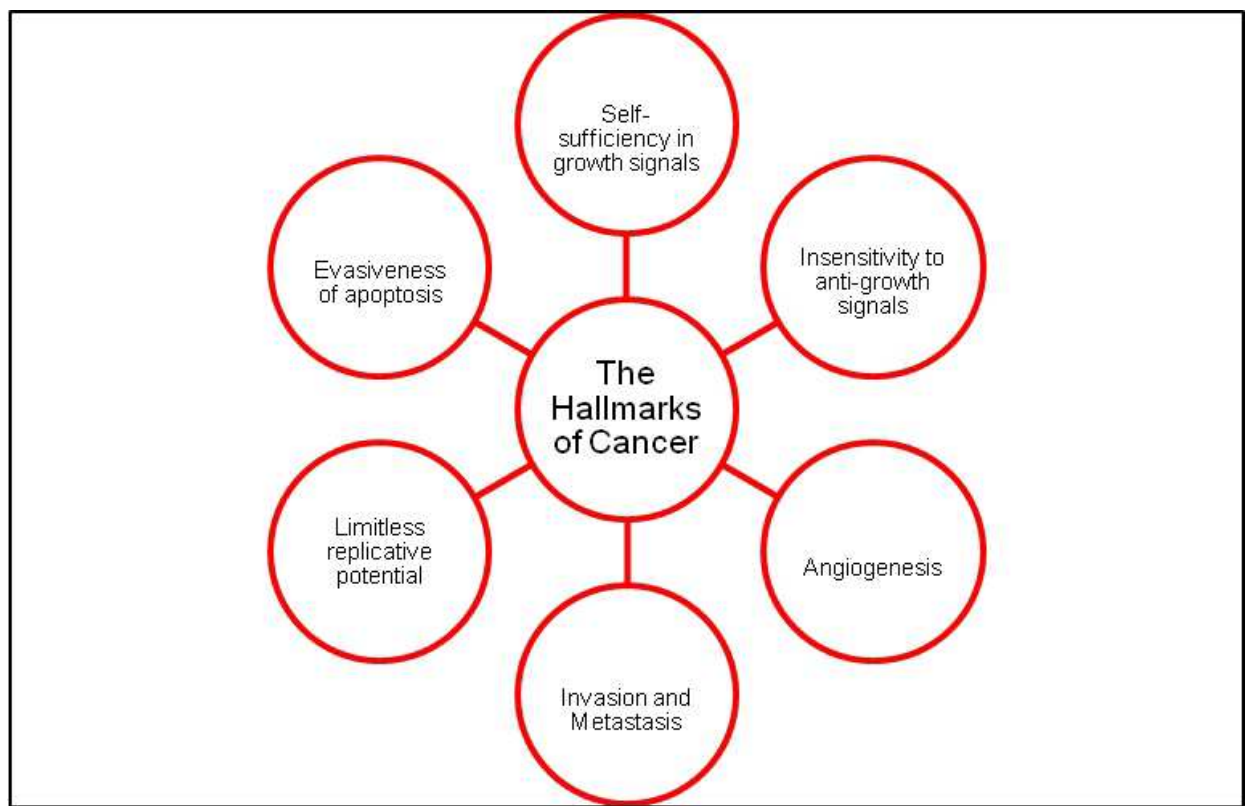Fig. 1. Three stages of carcinogenesis

Fig. 2. The Hallmarks of Cancer (Hanahan & Weinberg, 2000)

2. Prostate cancer

Prostate is a gland in the male reproductive system that is responsible for the production and storage of seminal fluid. The normal adult human prostate is about the walnut size and is located at the neck of the urinary bladder and surrounds part of the urethra. Prostate cancer develops when its semen-secreting prostate gland cells are transformed into cancer cells, and has been classified as adenocarcinoma. Prostate cancer is one of the most commonly diagnosed cancers in men and it is the second leading cause of cancer death after lung cancer worldwide. The incidence and mortality rates of prostate cancer are increasing in Asia as well as in the United States over the past few decades (National Cancer Institute [NCI], 2011).

2.1 Symptoms & diagnosis

Prostate cancer will cause male patients to experience difficulties during urination such as nocturia, hematuria, dysuria, and will also interfere with sexual functions and performance. The high mortality rate in prostate cancer patients is due to late detection as prostate cancer is usually asymptomatic or the symptoms appear only during the advanced stage of disease. About 50% of prostate cancer patients are usually diagnosed with bone metastasis. Table 1 shows several tests for prostate cancer diagnosis in male patients by doctor (NCI, 2011).

Test	Description
Biopsy	Removal of small pieces of prostate tissue through rectum (transrectal) or skin between scrotum and rectal (transperineal) for microscopic examination by urologist, oncologist and/or pathologist
Digital rectal examination	Detect prostate gland abnormalities such as lumps
Transrectal ultrasonography	High energy ultrasound from probe in the rectum is enable to create a picture (sonogram) of the prostate gland to examine its abnormalities
Prostate tumour markers	Detection the abnormal levels of prostate specific antigen (PSA) in blood

Table 1. List of test for the diagnosis of prostate cancer (Source: NCI, USA)

2.2 Stages

It is important to determine the stage of prostate tumour in order to plan an effective treatment in patient (Table 2). This is because there is a differential response to treatment in the different stages of this cancer. To determine accurately the stage of prostate tumour in patients, different tests are conducted and all information will be gathered from CT (computerised tomography) scan, MRI (magnetic resonance imaging), PSA (prostate-specific antigen) test and tumour biopsy (NCI, 2011).

Stage	Description
I	Cancer cells are found in prostate gland only and PSA < 10
II	Divided to 2 categories;
IIA IIA	Found in one half or less than one lobe and PSA < 20 Found in both lobes of prostate gland and PSA < 20
III	Spread beyond the outer layer of prostate gland and may spread to seminal vesicle, PSA value is ranging from 2-10.
IV	Spread to nearby or other organs such as rectum, bladder and bone, any level in PSA

Table 2. Stages of prostate tumour (Source: NCI, USA)

2.3 Treatments

Currently, there are four standard treatments for prostate cancer; watchful waiting, surgery, radiation therapy and hormone therapy. In watchful waiting, a patient will be closely monitored by doctors and no treatment will be given until symptoms start to appear. While

in surgery, prostate gland from patients will be removed before the cancer cells are able to spread to other organs. In radiation therapy, high energy X-rays will be used to kill cancer cells. Antiandrogens, estrogens and luteinizing hormone-releasing hormone agonists, are the few examples used in hormone therapy to stop the cancer cells by disrupt their growth and/or kill them. While other treatment includes chemotherapy and proton-beam radiation therapy which are used in conjunction with other standard treatments (NCI, 2011).

2.4 Problems

Prostate cancer is extremely hard to treat due to several distinct classes of tumour that exhibit different responses to treatment. Most of the synthetic anticancer agents such as doxorubicin mainly affect the fast-dividing cells of the body and cause side effects such as pain, nausea, vomiting, alopecia, and anaemia (CancerBackup, 2003). Nevertheless, prostate cancer cells are derived from normal cells and thus, cancer cells have the ability to maintain the usual physiological functions to protect themselves from anything that can cause cell destruction. Estramustine and mitoxantrone are the only two drugs approved by the US FDA for prostate cancer treatment; however, neither showed to increase lifespan of patients (NCI, 2011).

Therefore, the development of resistance to anticancer drugs by cancer cells is a major problem in cancer therapies and has resulted in a high mortality rate in prostate cancer patients. About 50% of male prostate cancers patients are diagnosed at advanced stage with metastasis into their bone (Uehara et al. 2003; Koeneman et al. 1999). Thus, tumour metastasis is a major cause of morbidity and has become a major challenge for a successful treatment (Hung et al. 2009). Currently, there is no effective treatment for prostate cancer, so intense research is required to obtain new anticancer agents for this cancer.

3. Natural products

Herbs and plants-derived medicines have a long history of use in the various treatments and now, they still remain as important sources for the development of anticancer drugs. More than 3000 plants species have been reported to be involved in the development of anticancer drugs (Shoeb, 2006). The exploration of anticancer agents from plant sources has started since the 1950s. Since then, extensive research and investigations have been conducted and lead to the discovery and development of several anticancer agents derived from plant such as taxol, vinblastine and vincristine (Cragg & Newman, 2005). From 1940-2006, more that 40% of drugs in the market are anticancer agents and 65% of these anticancer drugs mimic natural compounds.

The development of chemoresistant of cancer cells to anticancer drugs have resulted high mortality in prostate cancer patients. Thus, scientists have begun to focus on natural-product as alternative to produce new therapeutic agents in prostate cancer treatments. Although, no new plant derived clinical anticancer agents have been launched in market recently, but a number of agents are being tested in the preclinical stage such as thapsigargin (Cragg & Newman, 2005).

3.1 Thapsigargin (TG)

Thapsigargin is a plant-derived anti-tumour agent, isolated from Thapsia garganica L (Familly Apiaceae), collected in Mediterranean island of Ibiza. TG can evoke apoptosis in prostate cancer cells through the disruption of Ca^{2+} intracellular levels. In addition, TG can completely inhibit tumour growth in prostate tumour bearing nude mouse without causing

significant toxicity signs. Currently, TG is tested in preclinical stage against prostate cancer (Cragg & Newman, 2005).

3.2 Turmeric (curcumin)

Turmeric or known as curcumin is derived from the dried root of a plant (Curcuma longa L.) of the ginger family. It possesses a variety of pharmacological effects including anti-oxidant and anti-inflammatory activities. It has also been reported to possess anti-cancer effects through its inhibition on proliferation and apoptosis induction in wide variety of cancer cell lines including breast, lung and prostate cancer cells. The apoptosis induction is due to activation of pro-apoptotic proteins (Bax and Bak) and caspases involved in prostate cancer cells by turmeric. Currently in human clinical trials, turmeric is administered at dose up at 10g/day without causing any toxicity signs (Cragg & Newman, 2005; Kuttan et al. 1987).

4. Phyllanthus

The plant of the genus *Phyllanthus* is a small annual plant and is widely distributed throughout the tropical and subtropical regions of world (Lee et al. 1996). This plant has a long history in medical herbalism system to treat kidney and urinary bladder disturbances. Thus, substantial studies on *Phyllanthus* regarding their chemistry, pharmacological activity and clinical effectiveness have been carried out. The extracts of these plants have been reported to have pharmacological effects such as antiviral activity against hepatitis B and related hepatitis viruses, anti-bacterial activity, anti-hepatotoxic or liver-protecting activity, and hypoglycaemia properties (Etta, 2008; Mazumder et al. 2006; Kloucek et al. 2005; Ott et al. 1997; Lee et al. 1996; Blumberg et al. 1990; Venkatswaran et al. 1987).

The anticancer effects of the genus *Phyllanthus* has been reported in a few papers, for instance *P. amarus* protects the liver from hepatocarcinogenesis and the root extract of *P. acuminatus* exerts growth inhibition in murine P-388 lymphocytic leukemia and B-16 melanoma cell lines (Pettit et al. 1990; Powis & Moore, 1985). Thus, it is believed that the plant genus *Phyllanthus* might possess anticancer properties against prostate cancer as well.

Fig. 3. *P.urinaria* (Fito Pharma 2011), *P.amarus* (Siddha Global, 2010) and *P.niruri* (Nova Laboratories Sdn Bhd, 2011).

4.1 Bioactive compounds

"Bioactive compound" is a general term to describe the nutritional constituents that are contained in plants. These compounds are intensively studied and various reports from numerous types of epidemiological and case controlled studies have linked their effects on

health such as cardiovascular disease and cancer. Many bioactive compounds have been discovered and classified in different groups based on their chemical structure and function (Kris-Etherton et al. 2002). For example, phenolic and flavonoid compounds are found in all plants such as vegetables and fruits.

For the detection of bioactive compounds contained in *Phyllanthus*, HPLC (High Performance Liquid Chromatography) and MS-MS (Tandem mass spectrometry) are often used. These methods allow the separation and identification of compound from in *Phyllanthus* extracts. Various bioactive compounds have been identified from different species of *Phyllanthus* and are listed in Table 3. Most of these bioactive compounds such as gallic acid, gereniin and rutin, possess antioxidant and anticancer effects.

4.2 Anti-proliferative effects

One of the hallmarks of cancer is uncontrolled proliferation. Thus, the anti-proliferative effect of a substance is referring to the ability of the substance to halt cancer growth by inhibiting/killing cancer cells. The anti-proliferative effects of *Phyllanthus* have been detected in different cancer cell lines including breast, lung, melanoma, liver, lung, leukemia and prostate (Lee et al. 2011; Tang et al. 2010; Huang et al. 2006, 2004, 2003; Sureban et al. 2006). The water extract of *P.urinaria* have been found to possess a selective anti-proliferative effect on leukemia cells without showing cytotoxicity on normal cells (Huang et al. 2004). Similar findings have also been reported by Lee et al (2011) and Tang et al (2010), where four plant species of *Phyllanthus* (*P.amarus, P.niruri, P.urinaria* and *P.watsonii*) does possesses selective anti-proliferative effects on four different cancer cells; MeWo, A549, MCF-7 and PC-3 cell lines, without cytotoxic effects on their respective normal cells. In addition, the methanolic extracts of these plants were proven to have better anti-proliferative effects on cancer cells than aqueous extracts as their effects were exhibited at a relatively low dose. Several studies reported that the effectiveness of organic-soluble compounds in inhibiting or being lethal to cancerous cells is because most bioactive compounds are more likely to dissolve in organic solvents such as ethanol and methanol and only partially dissolve in polar solvents such as water (Wu et al. 2009; Saetung et al. 2005; Ojala et al. 2000). The anti-proliferative effects of *Phyllanthus* could be due to the presence of different bioactive compounds such as galic acid, rutin, geraniin and quercetin. Majority of polyphenol compounds found in *Phyllanthus* plant possesses anti-proliferative effects. One such example is gallic acid being found to have anti-proliferative effects on several cancer cells (Huang et al. 2009; Zhong et al.2009). Their anti-proliferative effects are always associated with their naturally antioxidant activity. The role of these polyphenol compounds on cancer have well documented as their can reduces the chance of cancer development by prevent mutation to occur in normal cells, which cause from free radicals. Due to their protective role on normal cells, the anti-proliferative effects on these cells are diminished.

4.3 Cell cycle inhibitor

Cell is the building block of all living things in this world. The cell cycle is a critical regulator that controls proliferation and growth of a cell. Cell cycle is the series of events that leads the cells to divide and replicate. This biological process involves a sequence of molecular events to ensure correct transmission of the genetic material to subsequent generations. Error in this transmission will lead the cells to either undergo checkpoints to correct the error or cause cell death. Defects in regulation of cell cycle will lead modification (mutation) in genetics of the cell and cells which leads to tumorigenesis. An uncontrolled proliferation

and the ability to evade of apoptosis by mutated (cancer) cells are the hallmarks for the development of cancer.

Compound	*Phyllanthus* species	References
Gallic acid	*P.amarus, P.niruri, P.urinaria, P.watsonii, P.emblica*	Lee et al. 2011; Tang et al. 2010; Zhong et al.2009; Huang et al. 2009
Corilagen	*P.amarus, P.niruri, P.urinaria, P.watsonii*	Lee et al. 2011; Tang et al. 2010; Zhang et al. 2004
Geraniin	*P.amarus, P.niruri, P.urinaria, P.watsonii*	Lee et al. 2011; Tang et al. 2010; Zhang et al. 2004
Caffeolquinic acid	*P.amarus, P.niruri, P.urinaria, P.watsonii*	Lee et al. 2011; Tang et al. 2010;
Rutin	*P.amarus, P.niruri, P.urinaria, P.watsonii*	Lee et al. 2011; Tang et al. 2010
Quercetin	*P.amarus, P.niruri, P.urinaria, P.watsonii, P.orbiculatus*	Tang et al. 2010; Huang et al. 2009; Nara et al., 1977
Galloylglucopyronoside	*P.amarus, P.niruri, P.urinaria, P.watsonii*	Lee et a. 2011; Tang et al. 2010
Phyllaemblic acid	*P.emblica*	Zhang et al. 2004
Chebulagic acid	*P.emblica*	Zhang et al. 2004
7V-hydroxy-3V,4V,5,9,9V-pentamethoxy-3,4-methylene dioxy lignan	*P.urinaria*	Giridharan et al. 2002
Astragalin	*P.niruri, P.urinaria, P.orbiculatus*	Nara et al., 1977
Phyllanthine and hypophyllanthine	*P.amarus*	Islam et al. 2008; Figueira et al.2006
Ellagic acid	*P.urinaria*	Huang et al. 2011, 2009

Table 3. Bioactive compounds in *Phyllanthus* species

Targeting the cell cycle could be an approach for anticancer agents to halt the uncontrolled proliferation of cancer cells and initiate them to undergo apoptosis (Sharpio & Harper, 1999). The cytotoxic effects of *Phyllanthus* extracts on growth inhibition against skin melanoma and prostate cancer cells in their cell cycle could partially explain their mode of activity.

Phyllanthus have been found to inhibit cancer growth by arresting the cancer cells at different cell cycle phases which later initiates them to undergo apoptosis. *Phyllanthus* had been found to arrest of different type of cancer cells in different phases. Both the aqueous and methanolic extracts of four different species of *Phyllanthus* (*P.amarus, P.niruri, P.urinaria* and *P.watsonii*) have been reported to induce G1-phase arrest in PC-3 cells (Tang et al. 2010). Arrested in G1 phase indicated that *Phyllanthus* extracts might disrupt the protein synthesis that required for PC-3 cells to enter next phase of cell cycle. In addition, these *Phyllanthus* species also disrupt the DNA synthesis on malignant melanoma MeWo cells by arrested them in S-phase during their cell cycle (Tang et al. 2010). Besides that, these findings reveal that the growth arrest of *Phyllanthus* treated PC-3 and MeWo cancer cells is accompanied by an accumulation of apoptotic cells. When the cells are arrested at a particular phase, they lose their uncontrolled proliferation properties and the cells will be initiated to undergo apoptosis. Although there are different polyphenol compounds contained in *Phyllanthus*, but they are believed to exhibits similar effects on cell cycle and led to apoptosis induction in treated cancer cells. For example, gallic acid and quercetin induces G1 phase cell cycle arrest and apoptosis in cancer cells by inactivate the phosphorylation of cdc25A/cdc25C-cdc2 via ATM-Chk2 activation, reduces cyclin D production and activate caspase-3 activity (Suh et al. 2010; Agarwal et al. 2006).

4.4 Apoptosis inducer

There are two main cell deaths; necrotic and apoptotic processes. Apoptosis is one of the main types of programmed cell death (PCD) that occurs only in multicellular organisms to eliminate damaged or unneeded cells without local inflammation from leakage of cell contents. It does provide a balance between cell proliferation and elimination, as a part of homeostasis. Apoptosis involves a series of biochemical events, which leads to distinct characteristics of cell morphology and death such as cell shrinkage and membrane blebbing. Necrotic cell death is characterized by cellular swelling, plasma membrane rupture, cell lysis and induction of inflammation around the necrotic cells due to leakage of the intracellular contents (Chen and Wang, 2002).

Dysregulation of the apoptotic process in human body can disrupt the equilibrium between cell's growth and death and this dysregulation implicated in a variety of diseases states. Acquired immunodeficiency syndrome (AIDS) and neurodegenerative diseases such as Alzheimer's disease are examples of diseases due to acceleration of cell death rates. Conversely, an inappropriate low rate of apoptotic process can give rise to autoimmune disorders or cancer (Fadeel & Orrenius, 2005). The genetic abnormalities of cancer cells are able bypass the apoptosis and escape from cell death. Thus, evasiveness of apoptosis is a hallmark of cancer and is critical for cancer development and the survival of a tumour cell (Vincent & Gatenby, 2008).

The regulations of apoptosis on cancer cells are always associated with caspases activation. Caspases (cysteine-aspartic proteases or cysteine-dependent aspartate-directed proteases) are a family of cysteine proteases that play essential roles in apoptosis. There are two types of apoptotic caspases: initiator and effector caspases. Initiator caspases (caspase-2, -8, -9 and -10) are responsible to activate the effector caspases which initially are in an inactive pro-form. The active effector caspases (caspase-3, -6 and -7) will in turn cleave other proteins to initiate the apoptotic process to occur. In addition, these caspases will also activate other degradative enzymes such as DNases, which cleave the DNA into fragments in an apoptotic cell.

The apoptotic inducer ability of *Phyllanthus* plant on various types of cancer cells has been identified (Lee et al. 2011; Tang et al. 2010; Huang et al. 2009; Zhong et al. 2009). Different

species of *Phyllanthus* plant has been shown to trigger activation of caspase-3 and -7, which later lead to apoptosis induction in treated cancer cells. These reveals that *Phyllanthus* can restore the apoptosis function of different cancer cells besides halting the uncontrolled proliferation of cell (Lee et al. 2011; Abhyankar et al. 2010; Ngamkitidechakul et al. 2010; Tang et al., 2010; Huang et al. 2009, 2004, 3003).

Different species of *Phyllanthus* induce apoptosis in human cancer cells in different ways are noticed. This could be due to various bioactive compounds contained in *Phyllanthus* and might use different pathways to induce apoptosis in cancer cells. For example, *P.amarus* induces apoptosis in human breast cancer cell line, MCF-7, by increasing their levels of intracellular reactive oxygen species (ROS) and decreased mitochondrial membrane potential (MMP). Besides that, *P.amarus* induces expression of caspase-3 and down-regulates expression of Bcl-2 to allow apoptosis in treated cancer cells. The down-regulation of the antiapoptotic family, Bcl-2 was also noticed in Lewis lung carcinoma cells after being treated with a different species of *Phyllanthus*, *P.urinaria*. In addition, *Phyllanthus* was also shown to induce TNF-α production and inhibit expression of other antiapoptotic genes including IL-8 and COX-2 in human hepatocarcinoma cells. Various bioactive compounds contained in *Phyllanthus* extract are believed to increase efficiency of *Phyllanthus* to induce apoptosis in cancer cells.

4.5 Anti-metastatic effect

Once cancer cells have transformed into malignant, they would have acquire metastatic in which the cells can detach from primary tumour and spread to other parts of the human body. Metastasis of cancer cells involves multistep processes and various cytophysiological changes, including disruption to adhesion interaction between cancer cells and the extracellular matrix (ECM) components. It also involves an over expression of proteolytic enzymes, such as matrix metalloproteinases (MMPs), which have the capability to degrade ECM components in the basal membrane of blood vessels. This allows the cancer cells to migrate and invade via the blood or lymphatic system into target organs and lead to the development of secondary tumours (Auerbach, 2000).

Therapeutic intervention strategies to prevent metastasis have an impact on cancer mortality. However, development of these therapies are requires a better understanding and knowledge in the biology and molecular events of the metastasis process. To achieve this, various of assays been conducted based on molecular events of tumour metastasis in human body.

The anti-metastatic properties of a compound can be defined by its inhibition on cancer cells' invasion and migration. *Phyllanthus* have shown reduced migration ability of treated cancer cells by reducing the number of treated cancer cells migrating through a 8-μm pore size transwell filter culture plate towards a growth factor (Lee et al. 2011; Ho et al. 2010; Ngamkitidechakul et al. 2010). The anti-migration effect of *Phyllanthus* was identified when gallic acid showed disruption on cancer cell-cell interaction in a mechanical scratch-wound cellular monolayer healing assay (Lee et al. 2011; Ho et al. 2010). In addition, *Phyllanthus* inhibited the invasion ability of cancer cells in a dose-dependent manner through the ECM as matrix barrier, which mimics the *in vivo* basement membrane of blood vessel (Lee et al. 2011; Ngamkitidechakul et al. 2010). All these indicate that *Phyllanthus* possess anti-metastatic effects by decrease cancer cells' migration and invasion abilities in dose-dependent manner.

The anti-metastatic effect of *Phyllanthus* was further noticed when *Phyllanthus* showed inhibition effects on different MMPs in different type of cancer cells. Table 4 below shows the inhibition effects of *Phyllanthus* on different MMPs.

MMP	*Phyllanthus*	References
MMP-1	*P.emblica*	Fujii et al. 2008; Chaudhuri et al. 2004
MMP-2	*P.urinaria*	Huang et al. 2010
MMP-3	*P.emblica*	Chaudhuri et al. 2004

Table 4. Inhibition on MMP members by different *Phyllanthus* species

4.6 Anti-angiogenesis effects

Angiogenesis is a process by where a new blood vessel is formed from pre-existing blood vessels. It is a normal and vital process in humans' growth and development, such as wound healing. In cancer, angiogenesis plays an essential role to its growth and development. In normal circumstances, solid tumour's size is around 1 to 2 mm^2 and not vascularized. However, when this tumour reaches beyond the 2 mm^2, oxygen and nutrients are hardly diffuse to the tumour and cause cellular hypoxia. This condition will lead to the onset of tumour angiogenesis (Folkman, 1971).

The balance between proangiogenic and antiangiogenic molecules is essential to maintain normal homeostasis in human body. Disruption of this balance would implicate to a variety of diseases states such as excessive (e.g. cancer and psoriasis) or inadequate (e.g. chronic wounds and stroke) angiogenesis. To date, angiogensis inhibitors have been reported to have less toxicity to normal cells and no development of drug resistance of cancer cells was notice such as bevacizumab, which been proven to improve the overall response including cancer time to progression, and survival rate. This makes the anti-angiogenic drugs highly advantageous over the conventional cytotoxic drugs. In addition, limited effectiveness of chemotherapy on advanced cancer has turn attention to these anti-angiogenic drugs and it is believed that these drugs will substitute the standard therapy (e.g. surgery, radiotherapy and chemotherapy).

Endothelial cells are involved in tumour angiogenesis by forming the linings of blood vessels, however, these tumour vessels have abnormal morphologies and are in immature forms. Thus, targeting these tumour vessels can be a promising target for antiangiogenic therapy (Auerbach, 2003). Tumour angiogenesis is critically important for tumour growth and metastasis with formation of new blood vessels to supply oxygen and nutrients to cancer cells and also to eliminate metabolic waste products (Auerbach, 2003). Thus, both tumour metastasis and angiogenesis are major cause of morbidity and they remain a challenge in cancer treatments.

Since the relationship between angiogenesis and cancer is clearly related where inhibition of angiogenesis could suppress/restrict tumour growth, this had attracted a strong interest from researchers worldwide to search for potential compounds that could inhibit angiogenesis. Although there are some successful anti-angiogenic drugs that have been commercialized and used in clinical and some in pre-clinical testing stage, but more effective and safer approaches are still required. The identification of new anticancer

compounds from nature has a long and successful history. Interestingly, there are some plants that possess proangiogenic (e.g. β-sitosterol and resveratrol) and antiangiogenic (e.g. camptothecin and combretastatin) properties and have been used in Traditional Chinese Medicine (TCM) from thousands of years. A wide range of plants contains compounds with angiogenesis modulating properties including pacific yew tree (e.g. Taxol) and Chinese tree *camptotheca acuminate* (e.g. camptothecin).

The anti-angiogenesis properties of *Phyllanthus* were supported by various scientific assays. *Phyllanthus* showed no cytotoxic effect on HUVECs (human umbilicial vein endothelial cells) as the cells' viability did not change. *Phyllanthus* also decrease the migration and invasion ability of HUVECs (Huang et al. 2006). The anti-angiogenic effect of *Phyllanthus* was noticeable where it inhibited capillary tube formation of endothelial cells on extracellular matrix, which mimics the *in vivo* lining of blood vessel. The anti-angiogenic effect of *Phyllanthus* was further proven by other *in vivo* studies where it could decrease the vessel density in a lung cancer animal model. Besides that, *ex vivo* studies using chick chorioallantoic membrane (CAM) assay also indicated the present of anti-angiogenesis in *Phyllanthus* (Huang et al. 2011).

4.7 Antitumour effects

The antitumour effects of *Phyllanthus* were reported in different cancer animal model including skin, liver and lung (Huang et al. 2006, 2003; Sancheti et al. 2005; Jeena et al. 1999). Currently, there are no toxicity of *Phyllanthus* on different experimental animal mice model were reported. Instead, *Phyllanthus* been found that possesses radioprotective activity by inhibit the myelosuppression and elevated the levels of antioxidant enzymes in the blood, liver and intestine and decrease the lipid peroxidation levels (Harikumar & Kuttan, 2007) in treated mice.

All studies indicate that *Phyllanthus* do possess antitumour effects on different tumour by reducing the tumour size and increase the lifespan of cancer animal models. The antitumour properties of *Phyllanthus* should be conducted in other cancer animal models such as prostate cancer to prove its antitumour properties to various cancers.

5. Conclusion

These findings revealed that *Phyllanthus* plants do possess anticancer activity in a selective manner towards cancer cells and initiate them to undergo apoptosis. The anticancer activity of *Phyllanthus* plant on prostate cancer cells was through its regulation on cancer cell cycle and apoptosis induction mediated via caspases activation. The anti-metastatic and anti-angiogenesis effects observed in *Phyllanthus* plant, indicate their potential in inhibiting the development of secondary tumour. Further investigations into the mechanism of anti-carcinogenic, anti-metastatic, anti-angiogenesis and apoptotic regulation properties of the herbal plant, *Phyllanthus* against prostate cancer cells is required. This may create an opportunity for the plants to not only be designed and developed as anticancer agents, but also as a dietary supplement for the prevention of cancer development. However, the preliminary *in vitro* data is insufficient and less convincing due to its limitation as all experiments are done in an artificial environment outside a human body. Thus, an *in vivo* study using experimental prostate cancer animal model is needed to determine the pharmacological and toxicological data as well as anti-tumour effect of *Phyllanthus*, to provide more information on the safe use and effectiveness of this plant.

6. Acknowledgment

7. References

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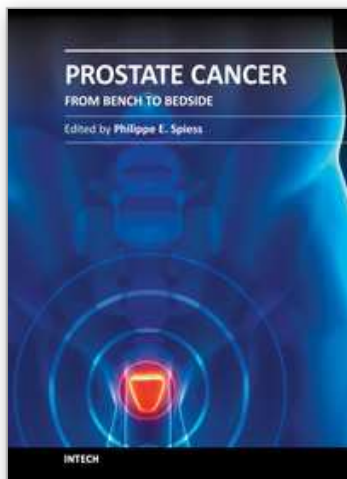
Prostate Cancer - From Bench to Bedside

Edited by Dr. Philippe E. Spiess

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