

CHAPTER 1

INTRODUCTION

1.1 General introduction

Cancer or malignant disease is a group of diseases characterized by uncontrolled growth and spread of abnormal cells. If the spread is not controlled, it can result in death. Cancer is caused by both external factors (tobacco, chemicals, radiation, and infectious organisms) and internal factors (inherited mutations, hormones, immune conditions, and mutations that occur from metabolism). These causal factors may act together or in sequence to initiate or promote carcinogenesis. The development of most cancers requires multiple steps that occur over many years. Certain types of cancer can be prevented by eliminating exposure to tobacco and other factors that accelerate this process. Other potential malignancies can be detected before cells become cancerous or at an early stage, when the disease is most treatable. Cancer is treated by surgery, radiation, chemotherapy, hormones, and immunotherapy (Garcia *et.al.*, 2007).

At present, cancer is the second leading cause of death in worldwide (12.5 % all of deaths), that only preceded by heart diseases (19.6 % all of deaths). In 2007, it is estimated that there will be more than 12 million new cancer cases worldwide, of which 5.4 million will occur in economically developed countries and 6.7 million in economically developing countries. The corresponding estimates for total cancer deaths are 7.6 million (about 20,000 cancer deaths a day), 2.9 million in economically developed countries and 4.7 million in economically developing countries. By 2050, the global burden is expected to grow to 27 million new cancer cases and 17.5 million cancer deaths simply due to the growth and aging of the population (Garcia *et.al.*, 2007). Cancer was the major cause of death in America, Europe, Asia and Thailand (Statistical annex, 2002) but in Hong Kong it was the leading cause of death in 1996-2001 (Stanley, 2001). Interestingly, cancer has been the first leading cause of death in Thailand for several years and the number of people died from cancer is still

increasing every year. Statistics from National statistical office of Thailand indicated that 52,062 Thai people died from cancer in 2006 (National statistical office, 2006).

There are several types of cancer which are the cause of deaths. In Thailand, liver cancer is the first common cancer in men (the third in worldwide). Most of primary cancers of the liver (PLC) are of epithelial cell origin, either from hepatocytes or intrahepatic bile duct cells. Hepatocellular carcinoma (HCC) arises from hepatocytes and accounts for 80% of all PLC (Khuhaprema & Srivatanakul, 2007). The second most common cancer in Thai male is lung cancer (the first in worldwide). Adenocarcinoma was the most common type of lung cancer followed by squamous cell carcinoma and small cell lung carcinoma (Martin, 2007). For women in Thailand, cervical cancer is the most common cancer (the second in worldwide). The main causal agents of cervical cancer are sexually transmitted almost certainly the human papillomaviruses (HPVs). Women infected with HPV-16 and HPV-18 have a 60-fold greater risk of developing cervical cancer than uninfected women; these two types have been identified in approximately 84% of cervical cancers. HPV-DNA was found in 82-91% of cervical carcinomas in Thai females (63-65% were HPV-16 and HPV-18), while HPV-DNA was found only 9.4% of normal cervical smears (Srivatanakul, 2007). The second most common cancer in Thai females is breast cancer (the first in worldwide). The age-specific incidence rates begin to rise at about 35 year and reach a maximum in 45 year, followed by a decline and a plateau. The most common histological subgroup of breast cancer is ductal carcinoma, which is found 76.4%-91.2% of cases, followed by other (8.6%-19.2%) and lobular carcinoma (0-6.4%) (Chaiwerawattana, 2007).

All these data indicate that these diseases are still a difficult problem on a worldwide basis and represent a major cause of death in both men and women. Surgery, radiation and cancer chemotherapy are major remedies for treating cancer patients. Cancer chemotherapy now plays an important role in the treatment of many malignancies either curative (by itself or used as an adjuvant to surgery and/or radiation) or in palliative care, depending upon the specific tumor situation (Carter, 1982). The objective of cancer chemotherapy is to kill cancer cells with as little damage as possible to normal cells (Halliwell & Gutteridge, 1988) so the discovery of anticancer agents must be related to novel molecular targets which should be defined

and developed for specific typical cancer cells but are less toxic to normal cells or have a unique mechanism of action for a specific type of cancer (Pezzuto, 1997).

Plant-based systems have a long history of use in traditional health care (Houghton, 1995). World Health Organization estimated that approximately 80% of the world's inhabitants rely mainly on traditional medicines for their primary health care and at least 119 chemical substances derived from 90 plant species can be considered as important drugs currently in use in one or more countries (Farnsworth *et al.*, 1985). 74% of these 119 compounds were discovered as a result of chemical studies directed at the isolation of the active substances from plants basic used from traditional medicine (Cragg *et al.*, 1997). Therefore, the usage of ethnopharmacology, or traditional use, is channeled for discovery of new biologically-active molecules (Houghton, 1995).

Thai traditional medicine (TTM) is a cultural heritage and indigenous wisdom which has helped to take care of the health of Thai people for over a thousand years. The basic principle of every branch of TTM is the knowledge of four elements or Dhatu (earth, water, wind and fire), their functions, and their interrelations, which affect the health of the individual. The imbalance and disharmony between the four body elements cause various diseases (Subchareon, 1998). The concept of cancer in TTM used by Thai traditional doctors is that it is caused by abnormal earth element or imbalance of the earth element in the patient body. The main cause is bad eating habits (e.g., too much, too little, or bad quality), emotion (e.g., sadness, depression, anger), environment (e.g., rapid change of temperature and climate), exercise e.g., (imbalance action and work), and excretion (e.g., abnormal excretion). These causes especially the excretion of waste products in the body, making the patient has much earth element or imbalance of the element. The patients with any of improper behaviors for a long time, especially incomplete excretion or accumulation of waste product in the body, tend to have abnormal cells develop into tumors. Preparations of Thai traditional medicines to treat illness include several herbs rather than a single herb. A herbal preparation for cancer treatment is commonly composed of many plants to balance the four basic elements of the body and to kill the cancer cells.

Benjakul is a Thai traditional medicine preparation which composed of five plants; *Piper longum* fruit, *Piper samentosum* root, *Piper interruptum* stem,

Plumbago indica root and *Zingiber officinale* rhizome, in equal proportions. It has been used for balanced health in Thai traditional medicine. Selective interviews with folk doctors of Southern Thailand and following their cancer patients by Itharat *et al.*, found that Benjakul was used as an adaptogenic drug, which was given to cancer patients before other drugs, resulting that cancer patients got well (Itharat *et al.*, 1998). The previous report of Benjakul preparation found cytotoxic activity against prostate cancer cells (Itharat *et al.*, 2004). In subchronic toxicity studies, Benjakul preparation was found harmless to tested animals (Chauwalitthamrong *et al.*, 1996).

Other previous studies of each plant which is component of Benjakul preparation are described below: Recently reports about *Piper longum* Linn. found that the alcoholic extract of fruits of *Piper longum* at a concentration of 500 µg/ml, showed 100% toxic against Dalton's lymphoma ascites (DLA) cells and 250 µg/ml against Ehrlich ascites carcinoma (EAC) cells. Its component, piperine also showed cytotoxic against DLA and EAC cells at a concentration of 250 µg/ml. Administration of alcoholic extract of *Piper longum* (10 mg/dose/animal) as well as piperine (1.14 mg/dose/animal) could inhibit the solid tumor development in mice induced with DLA cells and increase the life span of mice bearing Ehrlich ascites carcinoma tumor to 37.3 and 58.8%, respectively (Sunila & Kuttan, 2004). This extract was also shown to have antiangiogenic activity, using B16F-10 melanoma cell-induced capillary formation in C57BL/6 mice. Intraperitoneal administration of the extract (10 mg/dose/animal) significantly inhibited (50.6%) the number of tumor-directed capillaries induced by injecting B16F-10 melanoma cells on the ventral side of C57BL/6 mice (Sunila & Kuttan, 2006).

The previous reports about *Piper sarmentosum* Roxb. found that the methanol extract at concentration 200 µg/ml could inhibit RAJI of Epstein-barr virus which was activated by 12-O-tetradecanoylphorbol-13-acetate (Maurakami *et al.*, 1997).

Recently reports about *Piper interruptum* Opiz. showed no cytotoxic activity on KB cell (ED₅₀ = 58 µg/ml) but 90% ethanol extract at concentration 200 mg/kg can inhibit tumor cell in mice (Suffness *et al.*, 1988).

Recently reports about *Plumbago indica* Linn. found that the alcoholic extract from its root showed inhibitory effects for tumor growth on experimental

mouse tumors, S-180 solid tumor and Ehrlich ascites carcinoma *in vivo*. Intraperitoneal injection of 100 mg/kg of extract for 10 days starting from 24 hrs after intradermal inoculation of S-180 cells in BALB/c mice produced about 50% complete response. This extract was also shown to have inhibitory effect on growth of Ehrlich ascites carcinoma in Swiss mice at concentration 75 mg/kg (Devi *et al.*, 1994). The substance of *Plumbago indica* root, plumbagin, also exhibited anticancer effect in human non-small cell lung cancer cells (A549). It exhibited effective for cell growth inhibition by inducing cancer cells to undergo G2/M phase arrest and apoptosis (Hsu *et al.*, 2006).

Recently reports of *Zingiber officinale* Roscoe. found that the ethanolic extract of ginger exhibited anti-tumor-promoting effects in a mouse skin tumorigenesis model. Pre-application of extract to mouse skin significantly inhibited TPA-caused epidermal edema (56%) and hyperplasia (44%). In long-term tumor studies, topical application of extract 30 min prior to that of each TPA application to 7,12-dimethylbenz(a)anthracene-initiated SENCAR mice showed result as highly significant protection against skin tumor incidence (Katiyar *et al.*, 1996). The pungent substances which found in ginger rhizome as [6]-gingerol and [6]-paradol, showed anti-tumor promoting properties when studied in female ICR mice (Surh *et al.*, 1999). [6]-gingerol also had anti-angiogenic activity *in vitro* study and it also inhibited both the VEGF- and bFGF-induced proliferation of human endothelial cells as one cause of cell cycle arrest in the G1 phase. It also blocked capillary-like tube formation by endothelial cells in response to VEGF, strongly inhibited sprouting of endothelial cells in the rat aorta and formation of new blood vessel in the mouse cornea in response to VEGF (Kim *et al.*, 2005).

1.2 Rational of this study

In Thailand, many people have used traditional medicine as an alternative treatment for cancer (Subchareon, 1998). Folk doctors in Thailand have used Benjakul in cancer drug formulae (Itharat *et al.*, 1998). This traditional medicine are prepared by boiling the plant material in water or soaking in alcohol and are commonly used by Thai people to prepare the drug for oral intake. An investigation of

Benjakul which is an adaptogenic in a traditional drug formula to treat cancer patients. This preparation is used by folk doctors to treat cancer patient in Southern Thailand and it can improve the condition and quality of life. Surprisingly, there have been no studies on comparing biological activities of Benjakul preparation and each plant extract which were its components, especially cytotoxic activities against lung, breast, cervical and liver cancer. Therefore, the objective of this study are investigating cytotoxic activities of Benjakul and its ingredients extract against these types of cancer, which represent the main cause of death in Thailand. Bioassay guide fractionation will be used to isolate cytotoxic compounds with the highest cytotoxic activity against cancer cells, and to establish some structure-activity relationships for the isolated compounds. Moreover, the chemical fingerprints of Benjakul preparation and its ingredients will be investigated by high performance liquid chromatography for quality control of this preparation. It is hoped that this investigation will be provided the basic data of Benjakul preparation for cancer treatment, establish the guidelines for quality control of its extracts, and prove the cytotoxicity efficacy of Benjakul preparation with reliable and relatively consistent scientific data.

1.3 Aims of this study

1.3.1 Overall aims of this study

Overall aims of this research are to study on cytotoxicity and chemical fingerprints of ethanolic extracts of Benjakul preparation, to provide the basic data for the traditional doctor to treat cancer patients, and to be guidelines for quality control of the extracts.

1.3.2 Specific aims of this study

1.3.2.1 To study the cytotoxic activity of ethanolic extracts of Benjakul preparation and its ingredients against cancer and normal cell lines.

1.3.2.2 To isolate and characterize the cytotoxic compounds from the ethanolic extracts of Benjakul preparation by a bioassay-guide fractionation method.

1.3.2.3 To study the chemical fingerprints of the ethanolic extracts of Benjakul preparation and its ingredients by high performance liquid chromatography method.

1.3.2.4 To study the stability of active cytotoxic compounds that were isolated from the ethanolic extracts of Benjakul preparation under accelerate condition.