Chapter 1

Introduction

Cancer is a severe disease that causes the highest mortality rate in the world nowadays. In Thailand, the incidence rate of cancer has increased steadily since 1973. The Statistics Bureau, Ministry of Public Health reported that in 1973, cancer ranked sixth among all causes of death with 16.3 deaths per 100,000 people. In 1993, it was placed third with 45.0 deaths per 100,000 people and became the first leading cause of death in 1999 with 58.6 deaths per 100,000 people. In 2002, the cancer death rate was 73.3 per 100,000 people (Khuhaprema, 2004). The National Cancer Institute of Thailand reported that there were 10.5, 10.7, 10.7, 11.0, 10.1 and 10.1% newly diagnosed cancer cases from all new patients in 2002 to 2007 (Attasara, 2003; Attasara, 2004; Attasara, 2005; Attasara, 2006; Attasara, 2007; Attasara and Buasom, 2008). Nowadays, cancer still causes the highest death rate in Thailand, followed by coronary heart disease and accident. According to information of the National Cancer Institute, in 2007, the most common cancer in Thai male were bronchus and lung cancers (17.1%) following by liver and bile duct cancers (14.8%) and colon and rectum cancers (14.2%), respectively. In Thai female, the most common cancer was breast cancer (40%) following by cervix cancer (18.6%) and colon and rectum cancers (6.2%), respectively. (Attasara and Buasom, 2008)

Cancer affects lives and society and cannot be totally cured because the cancerous cells are increasingly less responsive to the body's normal regulatory machinery and are better able to invade normal tissues especially of the important organs such lung, liver, brain etc. The severity of cancer depends upon the type of cancer and (the extent of metastasis.) the spread of cancer from its primary site to other places in the body. Cancer is currently treated by surgery, radiation and chemotherapy. At present, more than 80 percent of the medicines used for chemotherapy derived from medicinal plants or traditional medicines (Farnsworth *et al*., 1985). Furthermore, 74% of 119 chemical compounds which are being recently researched for anticancer effects come from traditional medicines (Pezzuto, 1997). Therefore, traditional medicine knowledge leads to anticancer drug discovery

(Houghton, 1995). For example, the plants from *Podophyllum spp.* has been used to treat wart and cancer, and Epipodo-phyllotoxin isolated from Podophyllum resin has been used to be a precursor for semisynthesizing anticancer drugs such as etoposide and teniposide (Cragg *et al*., 1994).

Thai medicinal plants locally known as "Hua-Khao-Yen" have mostly been used in Thai traditional medicines (Ancient Medicine Association, 1962, 1978; Mutita, 1989; Pongbunrod, 1976; Traditional Lanna Thai Medicine, 1982). More than 2400 preparations of Hua-Khao-Yen have been registered at the Ministry of Public Health of the Thai government (Division of Medical Research, 1986). These preparations have been used to treat leprosy, venereal diseases, inflammations, bacterial infections and cancers. Moreover, Thai traditional doctors have used five species of "Hua-Khao-Yen" including *Dioscorea birmanica*, *Smilax corbularia*, *Smilax glabra*, *Pygmaeopremna herbacea* and *Dioscorea membranacea*, to treat cancers, AIDS, septicemia and lymphatic diseases. The extracts of these species are usually prepared by boiling with water or soaking with ethanol (Pongbunrod, 1976; Tungtrongjit, 1978). Among the five species, *Dioscoria membranacea* Pierre, called Hua-Khao-Yen-Tai, has been the most widely used to prepare Thai traditional anti-cancer medicines. By following the cancer treatments program of Thai traditional doctors in Songkhla province, it was found that the formula with *D. membranacea* could extend lifespan by 2-3 years for elderly patients and more than ten years for the young patients. The previous research showed that *D. membranacea* rhizomes was potently cytotoxic against cancer cells but less toxic to normal cells, making it possible to contribute to therapeutic effects. Its ethanolic extract exhibited high cytotoxic activity against COR-L23 lung cancer cell line, MCF-7 breast cancer cell line and LS-174T colon cancer cell line with IC_{50} of 6.2, 12.0 and 16.7 µg/ml, respectively while its water extract exhibited high cytotoxic activity against breast and colon cancer cell lines with IC_{50} of 5.5 and 15.6 µg/ml, respectively. Both extracts had no cytotoxic activity against SVK-14 keratinocyte normal cell line with IC_{50} more than 70 µg/ml, indicating the specificity of the *D. membranacea* extracts to only cancer cell lines (Itharat *et al*, 2004). Bioassay-guided isolation was used to isolate a novel and selective cytotoxic compound from *D. membranacea*, which is called dioscorealides B. This pure compound exhibited high cytotoxicity against breast and

lung cancer cell lines (IC_{50} = 0.92 and 1.59 µg/ml, respectively), less cytotoxicity to colon cell line (IC_{50}=5.26 µg/ml), and no cytotoxicity to keratinocyte normal cell line (IC_{50}=43.5 µg/ml) (Itharat *et al*, 2003). Ethanolic and water extracts of *D. membranacea* were tested for toxicity at Department of Medical Sciences, Ministry of Public Health and showed no acute toxicity to rats (LD_{50} = 9 g/kg and LD_{50} >25 g/kg, respectively) (Itharat and Ooraikul, 2007).

For these reasons, the aim of our studies was to develop the extraction processes of *D. membranacea* that gave extracts with high amount of dioscorealide B and high cytotoxic activity against breast cancer cell line**.** The isolated dioscorealide B was used as a quality control marker for validating our extraction methods in which chemical and biological activity characteristics of all extracts obtained by each method were determined. Our studies ultimately lead to development of anticancer drugs derived from *D. membranacea* in the future.

Objectives

1. Overall objective

Overall aims of this study were to develop the extraction methods that gave extracts with high yield of a cytotoxic compound, dioscorealide B, and high cytotoxic activity against MCF-7 and to determine stability of ethanolic extract under accelerated conditions.

2. Specific objectives

2.1 To isolate dioscorealide B from *Dioscorea membranacea* extract and use it as a marker for the quality control of the extract preparation

2.2 To develop the extraction methods that produce extracts with high content of dioscorealide B and high cytotoxic activity against MCF-7

2.3 To study the cytotoxic activity of *Dioscorea membranacea* extracts obtained by different extraction methods against the human breast adenocarcinoma cell line (MCF-7)

2.4 To study the stability of ethanolic extract of *Dioscorea membranacea* under accelerated conditions