CHAPTER I

INTRODUCTION

Liver cancer or hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide in terms of numbers of cases (626,000 or 5.7% of new cancer cases). The number of deaths is almost the same (598,000) because prognosis is very poor. It is therefore the third most common cause of death from cancer with only survival rates of 3% to 5% in cancer registries of the United States and developing countries (Parkin *et al.*, 2005). Many factors play a major role in the etiology of HCC, most importantly of them include hepatitis B and C viruses, alcohol, and aflatoxin exposure. More than 75% of the cases worldwide, and 85% of the cases in developing countries, are caused by both hepatitis B and C viruses. It is well established that a variety of cell cycle proteins play an important role in cancer etiology of liver cancer (Qin and Tang, 2002; Thomas and Abbrozzese, 2005).

Based on these observations, although several clinically used chemotherapeutic are drug preferably aiming at target molecule in HCC, their outcomes have not been promising including drug resistance and systemic toxicity specifically at the advance stage of this deadly malignancy (Chao *et al.*, 1998; Chun and Lee, 2004; Tien and Savaraj, 2006). These suggest that additional agents that are nontoxic in nature should be evaluated for their effects on chemopreventive in HCC cells. In this regard, natural products are a rich source of pharmacologically active compounds certain traditionally used herbal medicines and phytochemicals have been shown to chemopreventive and anticancer effect on HCC (D'Alessandro, Poma and Montalto, 2007).

Morus alba L. (Moraceae) or mulberry is cultivated in China, Korea, Japan, and Southeast Asian countries, including Thailand. Their leaves are used to feed silkworms (*Bombyx mori* L.) for producing silk. In recent years, the development of new uses for sericulture-related materials, such as silk, silkworms, and mulberry tea have been of great interest in the regions. Mulberry has been used traditionally to cure and prevent "Xiaoke" (diabetes) in Chinese medicine. *Cortex Mori*, commonly known as 'Sangbaipi' in Korea, 'Sanbaipi' in China or 'Sohakuhi' in Japan is the root bark of mulberry trees. It has been wildly used for anti-hyperglycemic, anti-inflammatory, diuretic, anti-tussive, expectorant, anti-cancer and antipyretic purposes in oriental medicine, whereas mulberry fruits are used as a tonic and sedative. Originating in China, Japan and Thailand, mulberry tea, a tea-like infusion of mulberry dried leave consumed for long time. A taste of mulberry brewing is for its pleasant flavor and medicinal benefits, some of which have scientific merit today (Yamatake *et al.*, 1978). Several studies have been reported for the components of mulberry leaves are the chemical groups of flavonoids, steroids, triterpenes, amino acids, vitamins and other trace minerals (Doi *et al.*, 2001; Yan, Wang, and Lu, 2004; Srivastava *et al*, 2006). In terms of their anticancer activity against cancer, a few studies shown that crude extract and flavonoid compounds from mulberry leaves are effective in the prevention of various cancers in cell culture models and their mechanisms of efficacy involving antioxidant activity, anti-metastasis, induction of apoptosis and induction of differentiation of cancer cell line (Kim *et al.*, 1999; Kim *et al.*, 2000; Nam *et al.*, 2002; Ahn *et al.*, 2006). However, the mechanisms underlying the chemopreventive activity of mulberry leaves are still unclear. The study of the effect of mulberry extracts on the regulation mechanisms at the molecular level may therefore clarify the

involved mechanisms. Our aim of this dissertation was to study of chemopreventive effect of mulberry leaves on a hepatoma cell line. Human hepatoma HepG2 which is a well-differentiated transformed cell line that meets all biochemical requirements was selected for the present study. This cell line has been widely used in biochemical and nutritional studies because it is considered one of the experimental models that more closely resembles the human hepatocyte in culture (Seow *et al.*, 2001; Mersch *et al.*, 2004; Xu, Duaz, and Ó Brien, 2004).

Rationale of the Study

In recent year, the concept of cancer chemoprevention has matured greatly; significant reversal or suppression of premalignancy in several sites by chemopreventive agents appears achievable. Experimental data on the antimutagenic and anticarcinogenic effects of teas have been conducted with water extract of green and black tea, or polyphenolic fraction isolated from tea (Katiyar and Mukhtar, 1997). Tea and several herbal infusions contribute to the major source of phenolic compounds in our diet (Atoui *et al.*, 2005). Such mechanistic studies, especially with group of polyphenols, include inhibition of free radical formation and lipid peroxidation, enhancement of antioxidant enzymes, induction of detoxifying enzyme in Phase II of Xenobiotic metabolism, the inhibition of ornithine decaboxlylase (ODC), induction of apoptosis, inhibition of regulatory proteins in cell cycle, inhibition of topoisomerase II and the inhibition of DNA-carcinogen binding and adduct formation (Kris-Etherton *et al.*, 2002; Robards *et al.*, 1999; Tiwari, 2001).

Antioxidant activity is a common action of bioactive compounds that show chemopreventive and therapeutic activity (Tsuda *et al.*, 2004; Tiwari, 2001). The antioxidant activity of herbs and spices caused mainly by phenolic compounds has

been demonstrated in many studies over recent years (Kris-Etherton *et al.*, 2002; Moure *et al.*, 2001). Several studies have been conducted for the presence and the antioxidant activity of tea and herbs but emphasis has been given to organic solvent extracts isolated from dried leaves. It has also been reported that inhibitory activities of polyphenol compounds in mulberry leaves against DPPH radical, superoxide radical, LDL oxidation (Choi *et al.*, 2002; Doi *et al.*, 2001; Kim *et al.*, 1999; Oh *et al.*, 2002; Zhishen *et al.*, 1999). A few studies have shown that crude extracts and flavonoid compounds from mulberry leaves are effective in the prevention of various cancers in cell culture models and their mechanisms of efficacy involving antioxidant activity, anti-metastatic, induction of apoptosis and induction of differentiation of cancer cell line (Kim *et al.*, 1999; Kim *et al.*, 2000; Nam *et al.*, 2002; Ahn *et al.*, 2006). In terms of their chemopreventive activity against HCC, there have not been reported about chemopreventive activity of crude extract from mulberry leaves against to HCC in cell culture models and also a little is known about the phenolic profiles, antioxidant activity in crude extracts from mulberry leaves. The purposes of this study were to determine total polyphenol content and to identity its active constitutes in extracts from mulberry leaves. Antioxidant capacity and the in *vitro* chemopreventive activity of mulberry tea against human hepatoma (HepG2) cancer cells were examined.

Objectives

This study has the following objectives:

1. To determine total polyphenol content and to identify quality profiles of its active constitutes in mulberry leaves (in form of hot water extracts and organic extracts).
2. To examine antioxidant capacity of the extracts by assessing their antioxidant capacity.
3. To investigate in *vitro* anticancer activities of extracts from mulberry leaves against human hepatoma cell line (HepG2), by assessing their cytotoxicity, effect on cell cycle distribution, induction of apoptosis, inhibitory effect on cyclin-dependent kinases inhibitor and inhibition effect on topoisomerase IIα.