# CHAPTER I
# INTRODUCTION

Liver disease is one of the major worldwide health problems caused by viral infections, poisons, drug abuse, and alcohol (Domitrovic, *et al.* 2013). Consumption of ethanol, a common organic solvent, can lead to several problems such as accumulation of triglycerides, inflammation, and alcoholic liver disease (ALD). The relation between ethanol and liver damage is mainly due to the fact that about 80% of received ethanol is metabolized in the liver (Faremi, *et al.* 2008). The enzyme alcohol dehydrogenase (ADH) in the liver converts ethanol to acetaldehyde. Cytochrome P450 2E1 (CYP2E1) assumes an important role in metabolizing ethanol to acetaldehyde at elevated ethanol concentrations. In addition, CYP2E1 – dependent ethanol oxidation may occur in other tissue where ADH activity is low. It also produces reactive oxygen species (ROS), which increase the risk of tissue damage. Acetaldehyde is metabolized mainly by aldehyde dehydrogenase (ALDH) in the mitochondria to form acetate and NADH. Acetaldehyde and acetate contribute to cell and tissue damage in various ways (Zakhari 2006). The toxic effect of ethanol in the liver has been extensively demonstrated in several animal and clinical studies. Thus, ethanol intoxication is a used model of liver damage.

HepG2 cells, a human hepatoma cell line, are considered a good model to study *in vitro* toxicity to the liver, since they retain many of the specialized functions which characterize normal human hepatocytes (Knasmuller, *et al.* 1998). HepG2 cells have been used for the study of liver disease (Cederbaum, *et al.* 2001), gene expression and transcription (Iyoda, *et al.* 2003), and mechanism of the action of drugs (Kim, *et al.* 2003). In particular, HepG2 cells are suitable indicators of compounds with genotoxic, antigenotoxic, cogenotoxics, and cytoprotective properties (Mersch-Sundermann, *et al.* 2004). This study aimed to investigate the anti-hepatotoxic effect of isolated compounds using human hepatoma HepG2 cell lines as the model and ethanol as the hepatotoxin, in order to relate *in vitro* anti-hepatotoxic activity.

Recently, there have been many studies focusing on the isolation and elucidation of biological active substances from medicinal plants for the treatment of liver diseases. For example, the aqueous-ethanolic extract of leaves of *Cassia occidentalis* L. has been shown to produce significant hepatoprotective effect on rat liver damage induced by paracetamol and ethanol (Jafri, *et al.* 1999). The ethanolic extract of *Tylophora indica* leaves possess a protective effect against ethanol-induced hepatotoxicity in rats (Gujrati, *et al.* 2007). In China, the aqueous extract of Zhi-Zi-Da-Huang decoction, a traditional Chinese formula comprising four crude drugs including *Gardenia jasminoides* Ellis, *Rheum officinale* Baill, *Citrus aurantium* L., and Semen Sojae Preparatum, has a potent hepatoprotective activity in alcohol-induced liver injury in rats (Wang, *et al.* 2009). In Thailand, the aqueous extract from *Thunbergia laurifolia* Linn. leaves, as known in Thai local name "Rang Chuet", protected mice from hepatic injury induced by ethanol (Chanawirat 2000). The aqueous extract of leaves of *T. laurifolia* has been shown to produce the hepatoprotective activity against ethanol induced liver injury in both primary cultures of rat hepatocyte and rats (Pramyothin, *et al.* 2005). The anti-hepatotoxic substances from *T. laurifolia* leaves have not yet been reported. Thus, in the present study aims to investigate the anti-hepatotoxic compounds from *T. laurifolia* on HepG2 cell line against ethanol induced toxicity.

In addition, hepatoprotective properties for the aqueous extract in the aerial parts of *Phyllanthus amarus* Schum. & Thonn. (locally known as Look Tai Bai) were demonstrated using animal models for paracetamol- (Wongnawa, *et al.* 2005) and ethanol-induced liver toxicity (Pramyothin, *et al.* 2007). Numerous studies have reported the hepatoprotective effect of phyllanthin, which is the major *P. amarus* bioactive lignan (**Figure 1**). Previous reports have indicated that phyllanthin exerts a hepatoprotective effect, through antioxidant activity, against ethanol-induced liver disease in a primary rat hepatocyte culture (Chirdchupunseree and Pramyothin 2010). Phyllanthin showed significant protection against $CCl_4$-induced hepatic damage by enhancing antioxidant activity (Krithika, *et al.* 2011). The present study was initiated to ascertain the anti-hepatotoxic activity of phyllanthin against ethanol-induced toxicity in a different model using HepG2 cells.

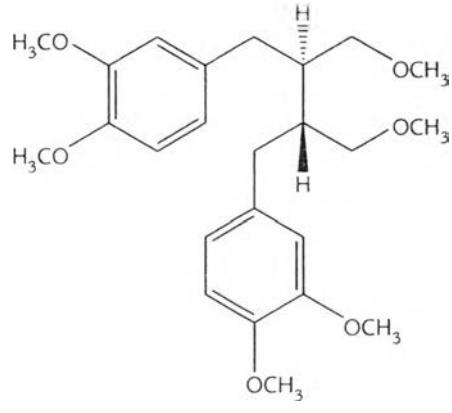


**Figure 1** Structure of phyllanthin

Standardizations of *P. amarus* plant materials and related commercial herbal drugs are essential to ensure their quality and bioactive phyllanthin. Some analytical techniques including, high performance liquid chromatography (HPLC) (Alvari, *et al.* 2011) and high performance thin-layer chromatography (HPTLC) (Annamalai and Lakshmi 2009) were previously developed for the quantitative analysis of phyllanthin from *P. amarus*. However, a rapid and inexpensive method for routine analysis of the major active compound, phyllanthin, in this plant is still preferred. Thin-layer chromatography (TLC-) image analysis method using a computer software technology has been developed and applied for quantitative analysis with precision and good accuracy (Phattanawasin, *et al.* 2012). Therefore, the aim of this study was to develop a rapid TLC-image analysis method for simple quantification of phyllanthin in *P. amarus* plant materials that can be used to determine the phyllanthin contents in commercial herbal drugs. The proposed method was to be validated in accordance with the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and the Association of Official Agricultural Chemists (AOAC) guidelines.

Meanwhile, the developed method has been suggested for the quantitative determination of bioactive compound in *T. laurifolia* leaves collected from different locations (Suwanchaikasem 2011).

Therefore, these two plants were selected for further investigation for their anti-hepatotoxic compounds. The data obtained in this study would be valuable information for its activity and would contribute to the knowledge on the chemotaxonomy of Thai medicinal plants.

The purposes of this research were as follows:

1. Isolation and elucidation of compounds from *T. laurifolia* leaves and investigation of the anti-hepatotoxic activity of isolated compound in ethanol-treated in HepG2 cell line.
2. Isolation and purification of phyllanthin from the whole plants of *P. amarus* and investigation of the anti-hepatotoxic activity of phyllanthin in ethanol-treated in HepG2 cell line.
3. Development and validation of a TLC-image analysis method for quantitative analysis of phyllanthin in the whole plant extracts of *P. amarus* collected from different locations in Thailand and in commercial herbal drugs.