CHAPTER I

INTRODUCTION

Centella asiatica (Linn.) Urban is a member of the Umbelliferae family. It is commonly known as Mandukparni or Indian pennywort in India and common name in Thai as Bua bok. The plant is a perennial, herbaceous creeper growing to 50 cm with fan shaped leaves (Cheng and Koo, 2000). It contains triterpenoids, principally asiatic acid, madecassic acid, asiaticoside and madecassoside (Inamdar et al., 1996). The whole plants of *C. asiatica* has been used as food ingredient, beverage and traditional medicine for the treatment of skin diseases, wounds, diarrhea or used as cardiotonic, diruretic etc., (สมภพ ประธานธุรารักษ์, 2546). Experimental studies revealed several pharmacological effects of *C. asiatica* extracts such as wound healing activity (Shetty et al., 2006; Shukla et al., 1999), protective effect on gastric ulcer formation (Cheng and Koo, 2000; Cheng et al., 2004), learning and memory enhancing effect (Gupta et al., 2003; Kumar and Gupta, 2002; Rao et al., 2005), antioxidant activity (Hamid et al., 2002; Jayashree et al., 2003; Zainol et al., 2003), antitumor activity (Bunpo et al., 2004; Kumar and Gupta, 2003; Siddique et al., 2008) and immunological activity (Jayathirtha and Mishra, 2004; Punturee et al., 2004; Wang et al., 2003, 2005). The results from those studies supported the traditional uses of *C. asiatica* as medicinal herb.

Cytochrome P450 (CYP), an enzyme system of phase I drug metabolism that found primarily in the liver. CYP enzyme is responsible for the metabolism of many drugs/substances, resulting in compounds that are generally less toxic and more hydrophilic, allowing for further conjugation reactions of phase II metabolism. Frequently, metabolism of parent compounds lead to the formation of the more toxic reactive metabolites (e.g. acetaminophen, carbon tetrachloride etc.). Human CYPs are grouped into 18 families and 39 subfamilies. The major CYP enzymes are CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4 (Manzi and Shannan, 2005). Various substrates such as caffeine, bupropion, warfarin, paracetamol, erythromycin, carcinogens etc., are metabolized by CYP. Ketoconazole, imipramine, miconazole, α-naphthoflavone, sulfaphenazole and grapefruit juice are among several drugs/substances known to inhibit CYP activities by destruction of

enzymes, or by complexing and thus inactivating enzymes (Coleman, 2005). In phase I metabolism, a polar functional group is introduced into the molecule rendering it a suitable substrate for phase II metabolism.

Phase II metabolism primarily results in either detoxification or activation, the later being the formation of products that are more active than parent compounds, Phase II metabolism includes glucuronidation, sulfation, glutathione conjugation, acetylation and amino acid conjugation. Glucuronidation, catalyzed by the UDP-glucuronosyltransferase (UDPGT) family of enzymes which is a major pathway of endogenous steroids, bile acid (Moon et al., 2006) and many exogenous compounds such as morphine (Collier et al., 2000). UDPGT was induced by polychlorinated biphenyls, hypolipidemic drugs (peroxisome proliferators), barbiturates (Iwata et al., 1992). Inhibition of glucuronidation is detected by 2-acetyl aminofluorene (Weisburger et al., 2002), 4-nitrophenol, probenecid (Sakai-Kato et al., 2004), silymarin (Andrea et al., 2005).

The sulfotransferase (SULT) catalyzes the sulfate conjugation of many hormones, xenobiotics such as phenols, alcohols, amines and thiol containing compounds. Various isoforms of SULT enzyme have been divided by their preferred substrates, molecular and gene structure such as phenol sulfotransferase, alcohol sulfotransferase, steroid sulfotransferase, and arylamine sulfotransferase. Phenol sulfotransferase was induced by farnesol, 4-OH-tamoxifen (Horn et al., 2005) and inhibited by salicylic acid, quercetin, curcumins and flavones (Zhang et al., 2007). Adenosine 3′,5′-bisphosphate, the analogue of 3′-phosphoadenosine-5′-phosphosulfate (PAPS) was shown to inhibit hydroxysteroid SULT activity (Lyon et al., 1981).

Glutathione *S*-transferase (GST) catalyzes the glutathione conjugation. This enzyme catalyzes the binding of a large variety of electrophiles to the sulfydryl group of glutathione, thus involved in the detoxification of a wide variety of harmful compounds. Allylsulfide, β-naphthoflavone, phenobarbital and pyrazol have been shown to induce GST activity, whereas carbon tetrachloride (Sheweita et al., 2001) and thonningianin A (Gyamfi et al., 2004) inhibit this enzyme.

NAD(P)H quinone oxidoreductase (NQOR, DT-diaphorase) has the ability to catalyzes the two electron reduction of quinones to hydroquinones and thereby protects cells against mutagenicity and carcinogenity (Iqbal et al., 2003). Methylene blue, 2,6-dichlorophenolindophenol and 1,4-naphthoquinone are used as substrates for NQOR (Dinkovo-Kostova et al., 2000). This enzymes is induced by probucol,

ascorbic acid, 3-(2)-tert-butyl-4-hydroxyanisole (BHA) (Elbakai et al., 2006), whereas dicumarol, *p*-chloromercuribenzoate, deaminothyroxine, atabrine and chlorpromazine inhibit this enzyme (Ernster, 1967). Effects of substrates, inhibitors and inducers on the activities of CYP and phase II drug metabolizing enzymes are associated with the issue of herb-drug interactions and the possibilities of any compound to increase or decrease risk of the individual to toxicity/mutagenicity/carcinogenicity from other xenobiotics.

A research group of the Faculty of Pharmaceutical Sciences, Chulalongkorn University has been granted the Integration Research Funding under the title of "Research and Development of the Standard Drug and Cosmetics Products from *C. asiatica* to Industry Manufacture". From 2003 to present, the standardized extract of *C. asiatica* (ECa 233) has been established with a constant ratio of the active ingredients of with possess a pharmacological effect. In the pharmacological study, ECa 233 was found to improve learning and memory deficit induced by bilateral common arteries occlusion in both Morris Water Maze and Step-down model. As motioned above, *C. asiatica* is an interesting herb with great pharmacological potential to be developed for medicinal purposes. However, safety assessment needed to be performed before this extract is given to in humans. In addition to the toxicological testing, drug interaction potential is also needed to be investigated.

If ECa 233 causes inductive and/or inhibitory effects on CYP and/or phase II drug metabolizing enzymes that play a key role in drug metabolism, this extract may cause the herb-drug interactions and/or cause an increase or decrease risk of toxicity/mutagenicity/carcinogenicity from other xenobiotics that are metabolized by the affected enzymes.

Effects of ECa 233 on phase I drug metabolizing enzymes, CYP *in vivo* in rats and *in vitro* using rat liver microsomes were investigated (Kulthong, 2007). The results showed that ECa 233 did not affect on the activities of CYP1A1, CYP1A2, CYP2B1/2B2, CYP2E1 and CYP3A4 *in vivo* study but caused a modest inhibitory effect on CYP2B1/2B2 with an IC_{50} of 523 μg/ml and 563 μg/ml using benzyloxyresolufin and pentoxyresorufin as substrates, respectively. However, the effects of ECa 233 on human CYP and phase II enzymes have never been studied. Thus, in this study effects of ECa 233 on the activities of human CYP enzymes were studied *in vitro* using recombinant human CYPs. In addition, effects of this extract on

hepatic phase II drug metabolizing enzymes were also determined *in vivo* using rat liver microsomes and cytosols prepared in study of Kulthong (2007).

Hypothesis

The standardized extract of *C. asiatica* (ECa 233) demonstrated an inhibitory effects on human CYP enzymes and caused an inductive/or inhibitory effects on phase II drug metabolizing enzymes in rat livers.

Study design and process

Experimental design: *in vitro* and *in vivo* studies.

1. Inhibitory effects of ECa 233 on the activities of human CYP enzymes *in vitro* using recombinant human CYPs (CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4)
2. A *in vivo* study
    2.1 Animal treatment with ECa 233
    2.2 Preparation of liver microsomes and cytosols
    2.3 Determination of phase II drug metabolizing enzymes (UDPGT, SULT, GST and NQOR) activities
3. Data analysis

Anticipated benefits from the study

1. A preliminary data demonstating an inductive and/or inhibitory effects of ECa 233 on major CYP and phase II drug metabolizing enzymes. This data provided the possibility of drug-drug interaction information of ECa 233 being taken simultaneously with other medicines metabolized by CYP and/or phase II drug metabolizing enzymes that were modulated by this extract.

2. In addition, this data would be useful to estimate the possibility of ECa 233 to increase/decrease risk of xenobiotic-induced toxicities, mutagenicities and/or carcinogenicities.