CHAPTER I

INTRODUCTION

Orthosiphon grandiflorus Bold, known locally as Yaa nuat maeo, Kidney tea plant or Java tea, is a medicinal plant in family Labiatae. *O. grandiflorus* is used traditionally for treatment of kidney diseases, dysuria with urinary stones, back and joint pain, diuretic, antihypertensive and antidiabetic (นันทวัน บุณยะประภัศร, 2529). It has been recommended that about 4 g of aerial parts of *O. grandiflorus* are ground, macerating with 750 ml of boiling water and the water is administered for the periods of 1-6 months for treatment of renal stones (สุนทรี สิงหบุตรา, 2542).

O. grandiflorus contains many chemical constituents such as diterpenes e.g. neoorthosiphols A and B, norstaminol A, orthosiphols A-J, orthosiphonones A and B, staminols A and B, staminolactones A and B, flavonoids e.g. cirsimaritin, eupatorin, 5-hydroxy-6,7,3',4'-tetramethoxyflavone, 6-hydroxy-5,7,4'-trimethoxyflavone, ladanein, pillion, rhamnazin, salvigenin, sinensetin, tetramethylscutellarein, 5,7,3',4'-tetramethoxyflavone, 5,7,4'-trimethoxyflavone, 7,3',4'-tri-*O*-methylluteolin and others e.g. acetovanillochromene, aurantiamide acetate, benzochromene, betulinic acid, caffeic acid, 2-caffeoyltartaric acid, coumarin, 2,3-decaffeoyltartaric acid, methylripariochromene A, oleanolic acid, orthochromene A, rosmarinic acid, β-sitosterol, ursolic acid, vomifoliol (Banskota, A.H., *et al.*, 2003).

Physiological and pharmacological properties of *O. grandiflorus* have been reported. *O. grandiflorus* aqueous extract was studied for treatment of renal stones. This plant caused little effects on serum and urine parameters related to stone risk factors as well as urinary pH. However, at high dose and long duration of administration, this extract caused an increase of urinary volume (อมร เปรมกมล และคณะ, 2545). This plant was found to increase urinary pH and promoters of renal stone such as oxalate, calcium (มยุรี เนิดน้อย

และวีระสิงห์ เมืองมั่น, 2535). In addition, *O. grandiflorus* caused a removal of renal stone and thus prevented urolithiasis in patients with renal stones (วีระสิงห์ เมืองมั่น และมยุรี เนิดน้อย, 2535). Chanarat, N., *et al.* (1997) studied the antihistamine effect of *O. grandiflorus*. They found that *O. grandiflorus* aqueous extract caused a relaxation of trachea smooth muscle and completely blocked the histamine-induced contraction of trachea smooth muscle. In addition, Chanarat, N., *et al.* (1997) and Kalaya Anulukanapakorn (2000) studied the hypoglycemic effect of *O. grandiflorus*. They found that *O. grandiflorus* aqueous extract caused a reduction of blood glucose but the effect was lower than the tolbutamide positive control group. *O. grandiflorus* hexane, aqueous-ethanol and aqueous-ethyl acetate extracts were shown to possess anti-herpes simplex virus type 2 strain Baylor 186 activity (สกุลรัตน์ รัตนาเกียรติ์, 2545). Methylripariochromene A, isolated from leaves of *O. grandiflorus*, caused a continuous decrease of systolic blood pressure and heart rate in conscious male stroke-prone spontaneous hypertensive rats (Matsubara, T., *et al.*, 1999). Moreover, diterpene derivatives obtained from methanolic extract of *O. grandiflorus*, caused a significantly production of nitric oxide in LPS-activated macrophage-like J774.1 cells (Awale, S. *et al.*, 2003; Nguyen, M.T.T. *et al.*, 2004), antiproliferative activities against highly liver metastatic colon 26-L5 carcinoma and human HT-1080 fibrosarcoma cell lines (Awale, S., *et al.*, 2002). Some diterpene derivatives obtained from *O. grandiflorus* exhibited a potent inhibitory activity against the inflammation induced by a tumor promoter, TPA (12-*O*-tetradecanoylphorbol-13-acetate). (Masuda, T., *et al.*, 1992).

Toxicological effects of *O. grandiflorus* have been reported. Acute and subacute toxicity study were performed in mice and rats using *O. grandiflorus* aqueous extract at doses of 0.5, 1 and 2 g/kg body weight. The median lethal doses (LD_{50}) in g/kg of the extract in animals after single intraperitoneal injection were 0.93 (male rats), 0.81 (female rats), 0.70 (male mice) and 0.84 (female mice). No lethal effect was found after single feeding of the extract up to 5 g/kg body weight in both rats and mice (ยุวดี วงษ์กระจ่าง และ คณะ, 2533). Regarding the subacute toxicity study, *O. grandiflorus* did not cause any changes in body weight, clinical blood chemistry and histopathology of major visceral

organs, i.e., kidney, liver, heart, lung and spleen as compared to the control group (ยุวดี วงษ์กระจ่าง และคณะ, 2533). Chronic toxicity study was performed in rats given an aqueous extract of dried aerial parts of *O. grandiflorus* at doses of 0.9, 9.0 and 18.0 g/kg/day for 6 months. The results showed that there were no difference in growth and food consumption between groups throughout the study. Generally, clinical signs revealed no abnormalities. Only at the dose of 18.0 g/kg/day that the platelet number was significantly increased. Serum sodium levels decreased in all treatment groups (ทรงพล ชีวะพัฒน์ และคณะ, 2536). The other chronic toxicity study was performed by giving the extract to rats at doses of 0.96, 2.40 and 4.8 g/kg/day per oral for 6 months. In general, no serious abnormalities was observed. Only at the highest dose (4.8 g/kg/day) that the animals exhibited a decrease of serum alkaline phosphatase and a significant increase incidence of hydrocalyx (นาถฤดี สิทธิสมวงศ์ และคณะ, 2542).

Effects of *O. grandiflorus* aqueous extract on hepatic cytochrome P450 (CYP) have not been reported. CYP is an important enzyme system in phase I metabolism which is catalytically responsible in the functionalization reactions of a variety of drugs, chemicals and environmental pollutants. Phase I metabolism *via* CYP may be detoxification or activation. Generally, CYPs in family 1, 2 and 3 play a key role in xenobiotic metabolism whereas the other remaining families of CYPs involve in endogeneous substance metabolism. Many CYP isoforms in family 1, 2 and 3 can be inhibited or induced by xenobiotics. Since *O. grandiflorus* has been traditionally recommended to be administered for months for the treatment of renal stone, repeated exposure of this herbal medical may affect (induce/inhibit) hepatic CYPs. Data from this study would provide a preliminary information of *O. grandiflorus* in the aspect of drug-drug interaction and the possibility of *O. grandiflorus* to increase/decrease risks of xenobiotic toxicity, mutagenesis and carcinogenesis. Therefore, in this study, effects of *O. grandiflorus* aqueous extract on hepatic CYPs including CYP1A1, CYP1A2, CYP2B1/2, CYP2E1 and CYP3A4 which are involved in drug metabolism and xenobiotic activation, were investigated using an *ex vivo* model in rats. In addition, blood samples of rats were also collected for determination of

hematology and clinical blood chemistry so as to obtain an additional data of subacute effect of this extract in rats.

Hypothesis

Subacute exposure of *O. grandiflorus* aqueous extract caused an induction and/or inhibition on hepatic microsomal CYPs as well as changes of clinical blood chemistry in rats.

Anticipated benefit form the study

A preliminary data of *O. grandiflorus* induction and/or inhibition effects on hepatic CYPs, especially CYP isoforms involved in drug metabolism and bioactivation of chemicals and environmental pollutants resulting in reactive metabolites. This would be useful to estimate the potential of drug-drug interaction when *O. grandiflorus* is taken simultaneously with other drug as well as the possibility of *O. grandiflorus* to increase and/or decrease risks of chemical-induced toxicities, mutagenicities and/or carcinogenicities. Effects of *O. grandiflorus* on clinical blood chemistry would be an additional data of subacute toxicity for this plant in rats.

Study design and process

1. Preparation of *O. grandiflorus* aqueous extract and chemical identification tests
2. An *ex vivo* study
   - 2.1 Animal dosing for 30 days
   - 2.2 Blood collecting
   - 2.3 Determination of clinical blood chemistry and hematology
   - 2.4 Preparation of liver microsomes
   - 2.5 Determination of microsomal protein concentrations, total CYP contents and CYP activities
3. Data collecting and analysis
4. Writing a thesis