CHAPTER V

DISCUSSION AND CONCLUSION

Results from this study showed that rats given *O. grandiflorus* aqueous extract at doses of 0.96 and 4.8 g/kg/day for 30 days did not modify (neither induce nor inhibit) the activities of CYP1A1, 1A2, 2B1/2, 2E1 and 3A. These CYP isoforms involved in carcinogenic and/or mutagenic activation of many chemicals and environmental pollutants. Thus, no induction effect of *O. grandiflorus* on these CYPs should be an advantageous feature of this compound regarding a potential increase risks of toxicity from xenobiotics *via* metabolic bioactivation. Chemicals/procarcinogens that are bioactivated by CYP1A1 including benzo(a)pyrene, dimethylbenz(a)anthracene, 6-nitrochrysene etc.; by CYP1A2 including 1-aminofluorene, 2- acetylaminoflurene, aflatoxin B_1 etc.; by CYP2B1/2 including aflatoxin B_1 etc.; by CYP2E1 including acrylonitrile, benzene, carbon tetrachloride, chloroform etc.; by CYP3A including aflatoxin B_1 etc. (Gonzalez, F. and Gelboin, H., 1994; Soucek, P. and Gut, I., 1992). In addition, no induction and inhibition effects on these CYP isoforms, CYP1A1, 1A2, 2B1/2, 2E1 and 3A, which are responsible for metabolism of many drugs are suggested to be beneficial characteristics of *O. grandiflorus* in term of drug-drug interactions if this plant and those drugs are administered concomitantly or at the close period of time. Examples of drugs that are metabolized by CYP1A2 include theophylline, paracetamol, phenacetin etc., by CYP2B6 (which is homologous to rat CYP2B1/2) include cyclophosphamide etc., by CYP2E1 include chlorzoxazone etc., by CYP3A include testosterone etc. (Taavitsainen, P., 2001; Berthou, F., 2001). However, *O. grandiflorus* aqueous extract given to rats at 4.8 g/kg/day was shown to significantly decrease total CYP contents as compared to the control group. A decrease of total CYP content that may be resulted from hepatotoxicity of *O. grandiflorus* was ruled out because serum hepatic enzymes (AST, ALT etc.) were not increased indicating that *O. grandiflorus* did not damage hepatic cells. If the extract did cause hepatic necrosis, all the CYP activities should be decreased. The other possible explanation was that several CYP isoforms that are

expressed in rat liver were not determined in this study such as several CYP isoforms in the subfamily of 2C and 2D (Soucek, P. and Gut, I., 1992). Taken together, a decrease of total CYP contents found in rats given *O. grandiflorus* at 4.8 g/kg/day may be contributed from the inhibition effect of this plant extract on other CYP isoforms that were not investigated in this study. Effects of *O. grandiflorus* on the activities of others CYP isoforms were then suggest to be explored.

To investigate effects of *O. grandiflorus* on clinical blood chemistry and hematology, it was shown that *O. grandiflorus* did not cause significant changes of these following clinical blood chemistry: AST, ALT, ALP, total bilirubin, direct bilirubin, total protein, albumin and globulin. This indicated that *O. grandiflorus* aqueous extract did not exhibit any toxic effects on the liver. Both doses of *O. grandiflorus* did not change serum creatinine, sodium and chloride while at the dose of 4.8 g/kg/day, a significant increase of BUN and serum potassium were observed. However, these parameters were still within or closed to the normal level of normal rats. This indicated that *O. grandiflorus* aqueous extract may exerted some slight effects on kidney when given at high dose. This was consistent to a previous chronic toxicity study that they found an increase incidence of hydrocalyx in male rats receiving *O. grandiflorus* at the dose of 4.8 g/kg/day for 6 months (นาถฤดี สิทธิสมวงศ์ และคณะ, 2542). An increase of serum potassium in rats given the extract at high dose (4.8 g/kg/day) may be due to either effect of the extract on the kidney or the presence of high potassium in the extract. Potassium was found with quite large amount in the extract (11.52% w/w of the extract). Regarding effects of *O. grandiflorus* on blood system, no significant changes were shown on these following hematological parameters: hematocrit, hemoglobin, platelet count, red blood cell count, RBC indices (MCV, MCH and MCHC), RBC morphology and white blood cell count. Even though at the dose of 4.8 g/kg/day, a significant decrease of lymphocyte and an increase of polymorphonuclear were observed, these parameters were still within or closed to the normal level of normal rats. This was somewhat difference from a previous study performed in female rat receiving *O. grandiflorus* aqueous extract at the dose of 4.8 g/kg/day for 6 months that did not show these changes but a significantly increase of platelet counts which was still in the normal

range (นาถฤดี สิทธิสมวงศ์ และคณะ, 2542). Slight difference between studies could be contributed from many factors such as source of the plant, the extraction procedure, sex and strain of rats, the environmental handling of animals, etc. Food and water intake of rats receiving both doses of *O. grandiflorus* aqueous extract were not significantly different from the control group thoughout the study period while this extract caused significant decrease of body weight gain at the dose of 4.8 g/kg/day. A decrease of body weight gain that may be resulted from decrease of food and water consumption in rats was ruled out. Some toxic effects of *O. grandiflorus* when given at high dose on other organs or systems that were not investigated in this study, may contribute to the decrease of rat body weight gain.

In general, *O. grandiflorus* is used traditionally for treatment of dysuria with urinary stones by macerating dried leaves of the plant with hot water and the obtained water is used. This is similar to the protocol that this study performed to yield the aqueous extract fraction. The aqueous extract fraction was also studied and shown to be pharmacologically active for diuretic and renal stone effects (มยุรี เนิดน้อย และวีระสิงห์ เมืองมั่น, 2535; วีระสิงห์ เมืองมั่น และมยุรี เนิดน้อย, 2535). Thus, aqueous extract fraction of *O. grandiflorus* was used in this study. Following the extraction procedure, percentage yield of the extract was 12.7% w/w which was somewhat different to other previous studies. In those studies, percentage yield of the extract was 17% w/w, 24% w/w and 9.6% w/w. (Englert, J. and Harnischfeger, G., 1991; ยุวดี วงษ์กระจ่าง และคณะ, 2533 and นาถฤดี สิทธิสมวงศ์, 2542). Difference of the percentage yield of the extract between studies could be contributed from many factors such as source of the plant, the extraction procedure, etc. *O. grandiflorus* aqueous extract was given orally to rats in 2 treatment groups at the doses of 0.96 and 4.8 g/kg/day which were equivalent estimated to 7 and 35 folds of the dosage recommended for traditionally used in human. Chemical identifications including preliminary test with color reaction tests (such as test with potassium permanganate TS and ferric chloride TS) , confirmatory test with thin layer chromatographic analysis and determination of potassium content were performed according to the method suggested by Pattamadilok, D., *et al.* (2002) before the extract was given to the animals. Preliminary test with color tests were used to detect the phenolic constituents. Confirmatory test with thin layer chromatographic analysis was

performed by comparing with the reference standards such as rosmarinic acid, caffeic acid and ursolic acid, the constituents that found in the aqueous extract of *O. grandiflorus* (ดวงเพ็ญ ปัทมดิลก และคณะ, 2545). The results showed that *O. grandiflorus* aqueous extract used in this study was composed of phenolic compounds *via* color reaction tests which were similar to the study reported by Pattamadilok, D., *et al.* (2002). However, the confirmatory test with thin layer chromatographic analysis could not detect ursolic acid in *O. grandiflorus* aqueous extract which was different from the study reported by Pattamadilok, D., *et al.* (2002). Ursolic acid in the extract may presented in a very small amount that could not be detected by the system of TLC used in this study. Normally, ursolic acid was poorly extracted by polar solvent such as water while better extraction could be shown if less polar or nonpolar solvent was used such as ethanol or hexane, respectively. Factors affecting the constituents in the plant were then interesting to be solved. This study was performed using an *ex vivo* model in rats which was the model that both inhibition and induction effects of CYP could be detected simultaneously. These model was detect irreversible inhibition effects of CYP while did not detect competitive inhibition effects of CYP. Generally, CYP induction requires repeated exposures to the compound for a period of time depending upon the type of compound such as 2, 3, 5, 7 days or more of consecutive exposures. In this study, *O. grandiflorus* aqueous extract was given to the animals for 30 days, the duration which was sufficiently enough to detect an induction effect as well as obtaining the subacute toxicity data from the same experiment.

In conclusion, subacute effects of *O. grandiflorus* aqueous extract on hepatic CYPs and clinical blood chemistry were studied in male Wistar rats. Two doses (0.96 and 4.8 g/kg body weight/day) of the extract were given orally to rats for 30 days compared to the control group given distilled water in the same manner. The results showed that *O. grandiflorus* aqueous extract caused a significant decrease of total CYP content while did not cause significant effect on the activities of CYP1A1, 1A2, 2B1/2, 2E1 and 3A. *O. grangiflorus* aqueous extract did not produce changes of clinical blood chemistry and hematology as following: hemoglobin, hematocrit, platelet count, white blood cell count, red blood cell count, RBC indices (MCV, MCH and MCHC), RBC morphology, AST, ALT, ALP, total

bilirubin, direct bilirubin, serum creatinine, total cholesterol, triglyceride, LDL-C, HDL-C, glucose, uric acid and electrolytes (sodium, chloride and calcium). However, at the dose of 4.8 g/kg/day, the extract caused slight effect on kidney with a significant increase of BUN and serum potassium. Effects of *O. grandiflorus* on other isoforms of CYP which were not investigated in this study were suggested for further investigation.