Indonesian J. Pharm. Vol. 25 No. 4 : 230 – 236
ISSN-p : 2338-9427
DOI: 10.14499/indonesianjpharm25iss4pp230

Research Article

INTERACTION STUDY: THE EFFECT OF *ORTHOSIPHON STAMINEUS* EXTRACT ON HUMAN CYTOCHROME P450

Purwantiningsih[1]*, Abas Hj Hussin[2]

[1]Dept of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Gadjah Mada University, Skip Utara, 55281, Yogyakarta, Indonesia
[2]Centre for Drug Research, Universiti Sains Malaysia, 11800, Penang, Malaysia

Submitted: 05-05-2014
Revised: 07-08-2014
Accepted: 21-09-2014

*Corresponding author
Purwantiningsih

Email :
purwanti_n004@yahoo.com

ABSTRACT

Herbal remedies are often used concomitantly with prescribed medication, which leads to an increase in the potential of herb-drug interactions. *Orthosiphon stamineus* is one of the popular herbal preparations that is traditionally used, especially as a diuretic or for the elimination of kidney stones. The inhibitory effect of *O. stamineus* extract on human cytochrome P450 isoforms, namely CYP1A2, CYP3A4, CYP2C9 and CYP2D6, has been determined using luminescent methods. The final concentrations of *O. stamineus* extract tested were 0.01, 0.1, 1, 10, 100 and 1000µg/mL. IC_{50} value was used to assess the modulation potencies for each CYP isoform. The extract showed a moderate inhibition towards CYP2C9 with the IC_{50} of 20.12µg/mL and showed IC_{50} of 49.90, 89.24 and 97.82µg/mL for CYP1A2, CYP2D6 and CYP3A4, respectively. These data suggest that *O. stamineus* extract may potentiate the herb-drug interaction via CYP inhibition.

Key words: *Orthosiphon stamineus*, inhibitory effect, CYP isoform

INTRODUCTION

Herbal preparations have been widely used in the world and the market has been rapidly growing in recent years. *Orthosiphon stamineus* Benth, belonging to the family Lamiceae, is one of the most popular medicinal plants in Southeast Asia, including Indonesia and Malaysia. *O. stamineus* is locally known as Misai Kucing or remujung, and has received great attention from researchers. The plant is used as an alternative medicine in Malaysia and has also been sold as a dietary supplement in recent years (Wiart, 2002). In many European countries, *O. stamineus* is consumed as a herbal tea to promote health due to its high antioxidant properties (Indubala and Ng, 2000).

O. stamineus contains several chemically active constituents; one of the most important classes of compounds is the phenolic group. Some researchers have isolated many phenolic compounds including lipophilic flavones, flavonol glycosides, and caffeic acid derivatives, such as 2,3-dicaffeoyltartaric acid and rosmarinic acid, which have been identified and quantified by High Performance Liquid Chromatography (Sumaryono *et al*., 1991). *O. stamineus* leaves contain polymethoxylated flavones, sinensetin, tetramethylscutellarein and 30-hydroxy-5,6,7,40-tetramethoxyflavone, as reported by Pietta *et al*. (1998). Other researchers (Tezuka *et al*., 2000) found the chemical constituents of *O. stamineus* to include caffeic acid derivatives, diterpene esters, triterpene saponins, flavonoids, antioxidants and volatile oil.

A number of interaction studies of *O. Stamineus* have been published. Chin *et al*. (2009) reported that methanol leaf extracts of *O. Stamineus* increased both UGT and GST activity in the diabetic rat liver. An *in vitro* inhibitory study of *O. Stamineus* on UGT isoforms showed that the extract inhibited UGT1A9, UGT1A1, UGT1A6 and UGT1A8 with an IC_{50} of 10.83, 24.65, 30.02 and 43.39µg/mL, respectively. However, the extract showed an IC_{50} of more than 50µg/mL for UGT1A3, UGT1A10, UGT2B7 and UGT2B15 activities (Ismail *et al*., 2010). Chin and Hussin (2011) reported that *O. Stamineus* increased aminopyrine metabolism by inhibiting protein kinase-A in female rat hepatocytes.

The use of herbal remedies that are often co-administered with prescribed drugs has led to an increase in herb-drug interactions (Zhou *et al*., 2004). These herb-drug interactions have a high prevalence but are often unknown, which signifies negligence of consumers with regard to reporting herb-drug interactions or adverse reactions when taking herbal medicines (Barnes *et al*., 1998). There are many reports that provide evidence for clinically important herb-drug interactions, where the concurrent use of herbal medicines with synthetic drugs is noted (Fugh-Berman, 2000).

The herb-drug interactions may occur with the use of *O. stamineus* and prescribed drugs since

the herb is commonly used to treat diabetes mellitus, hypertension and gout problems in many Southeast Asia countries (Wiart, 2002). The interaction may occur during the pharmacokinetic phase, which includes absorption, distribution, metabolism or excretion processes, and also in the pharmacodynamic phases (Izzo *et al*., 2002). Alteration of drug concentrations by the concurrent use of herbal medicines may occur through the inhibition or induction of hepatic drug-metabolizing enzymes, especially the cytochrome P450s (CYP) (Wilkinson, 1997; Ioannides, 2002; Zhou *et al*., 2003). The aim of this study was to examine the effect of *O. stamineus* on the activities of human CYP450 isoforms, especially CYP1A2, CYP2C9, CYP2D6 and CYP3A4.

MATERIALS AND METHODS

Reagents

The luminescence assay was performed according to Hanapi *et al.* (2010), using a 96-well microtiter plate using the P450-GLO™ Screening System from Promega®, USA (Thermo Scientific®, Finland). The screening systems included the four sub-types of CYP450 (CYP 1A2, 2C9, 2D6 and 3A4) and 4 different substrates were used in these experiments: luciferin 6' methyl ether (luciferin-ME) for CYP1A2 and 6' deoxyluciferin (luciferin-H) for CYP2C9, and the ethylene glycol ester of luciferin 6' methyl ether (luciferin-H EGE) and luciferin 6' benzyl ether (luciferin-BE) for CYP2D6 and CYP3A4, respectively. Each system contained the luciferin detection reagent, the CYP membranes and control membranes, the solution A and B of the NADPH regeneration system, 1M potassium phosphate buffer and luciferin-free water. Four known positive controls, sulfaphenazole, alpha naphtoflavone, quinidine and ketoconazole, were purchased from Sigma Chemicals, MO, USA.

Plant materials

The *O. stamineus* extract was provided by Prof. Zhari Ismail from the School of Pharmaceutical Sciences, Universiti Sains Malaysia. The extract was obtained according to the previous study. *O. stamineus* leaves were collected in the afternoon, from plants aged 30 to 45 days, white flowered plants and the voucher specimens of plant material were deposited at Bilik Herba, School of Pharmaceutical Sciences, Universiti Sains Malaysia. Methanol extraction of *O. stamineus* was performed by extracting the dried leaves (10g) with 100mL of methanol at approximately 40°C for 4 hours with continuous stirring. The extract was filtered through Whatman filter paper (No. 1), and then concentrated and spray-dried to obtain the crude methanol extract (Akowuah *et al*., 2004).

Luciferin standard curve preparation

Luciferin standard curve of each CYP was performed by preparing the D-luciferin stock solutions at concentration of 2mM. Then, a serial dilution concentration of D-luciferin standard (four times concentration) was made to obtain the standard solutions with final concentrations of 2μM, 0.4μM, 0.08μM and 0.016μM. The reaction mixture and reaction control mixture for each CYP (CYP1A2, CYP3A4, CYP2C9 or CYP2D6) were prepared at four times concentrations, while the NADPH regeneration system was prepared at a two times concentration. The reaction was started by adding 12.5μL of D-luciferin standards into a 96-well white opaque plate in the appropriate wells; in the 0μM D-luciferin wells, 12.5μL of luciferin-free water was added. The control reaction mixture (12.5μL) was then added and the plate was shaken by tapping the plate carefully. The reaction system was pre-incubated for 10min at 27°C and 25μL of CYP NADPH regeneration system was added to each well. The plate was tapped again and incubated for 30min at 27°C. The reconstituted luciferin detection reagent (50μL) was added to all of the wells, was briefly mixed and then incubated for 20min at 27°C to stabilize the luminescent signal. The signal was recorded using a plate-reading luminometer (Hidex Plate CHAMELEON®, Hidex Oy, USA) and the values were displayed as relative light units (RLU).

Enzyme assay

The enzyme assay was performed by adding the test compound of *O. stamineus* solution (12.5μL) with concentrations ranging from 0.01 to 1000μg/mL or the positive control solution ranging from 0.02 to 200μM to the wells of a microtiter plate. In the untreated wells, the test compound was replaced with the

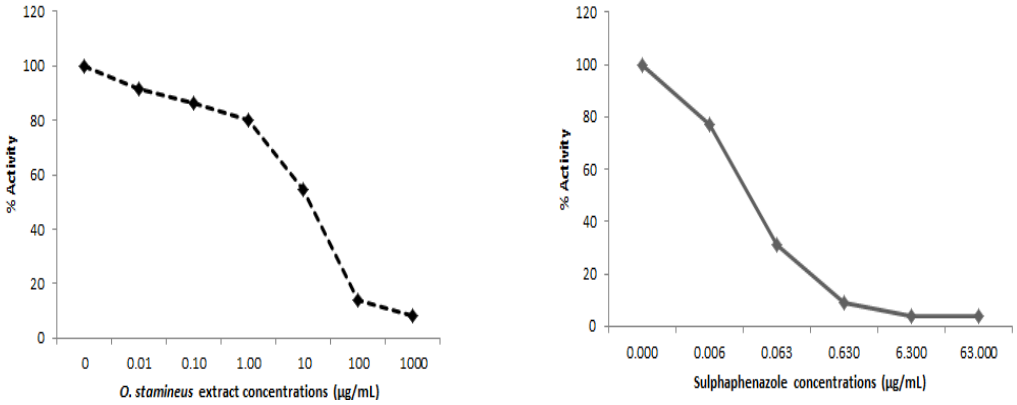

Figure 1. Effect of *O. stamineus* (A) and Sulphaphenazole (B) on the CYP2C9 activity.

12.5µL of luciferin-free water. Then, 12.5µL of the control reaction mixture was added and the reaction was continued as described in section "luciferin standard curve preparation".

Data analysis

The data are presented as percentage of activity compared to the blank group and the IC_{50} was determined to examine the inhibitory potency of *O. stamineus* extract and the positive control on cytochrome P450 enzyme activity.

RESULTS AND DISCUSSION

This interaction study used four human CYP450 isoforms namely CYP1A2, CYP3A4, CYP2D6 and CYP2C9 to examine the effect of *O. stamineus* and positive control on the enzyme activities using a luminescent assay. Alpha naphtoflavone, ketoconazole, quinidine and sulphaphenazole were used as positive control towards CYP1A2, CYP3A4, CYP2D6 and CYP2C9, respectively. In this assay, beetle luciferin was taken as a luminogenic P450 probe substrates. Beetle luciferin would be converted by different P450 enzymes to luciferin. Then, the luciferase enzyme will create light from the free luciferin as a substrate and supported by ATP and oxygen. The light produced is directly proportional to the activity of CYP450 enzyme and was measured using a microplate reader. Luminescent assay is more profitable as compared to the fluorescent assay, because there has no fluorescent interferences (Bosetti *et al.*, 2005)

Interaction results of *O. stamineus* and the positive control towards four CYP450 isoforms are shown in Figure 1-4, while the IC_{50} results are shown in Table I. The methanol extract of *O. stamineus* showed an inhibitory effect on CYP450 activities, with the highest inhibition towards CYP2C9 and has an IC_{50} at 20.12µg/mL. The *O. stamineus's* IC_{50} was greater if compared to sulphapenazole as positive control with an IC_{50} at 0.03µg/mL (Figure 1). The same phenomena were also found in the interaction results of *O. stamineus* extract towards CYP1A2, CYP2D6 and CYP2C9 activities with IC_{50} values of 49.90µg/mL, 89.24µg/mL and 97.82µg/mL, respectively. The positive control showed the IC_{50} values of 0.007µg/mL, 2.9µg/mL and 0.003µg/mL, correspondingly (Figure 2-4). These findings suggest that these plants showed a weak inhibition compared to the positive control. This may because the extract contained many compounds so that the interaction with each CYP450 was not specific.

The *O. stamineus* extract was reported to possess rosmarinic acid as the main component, with concentrations ranging from 5.1% to 29.9% of the total dry leaf weight; also, this extract contained 3'-hydroxy-5, 6, 7, 4'-tetramethoxyflavone, eupatorin and sinensetin, with levels ranging from 0.05% to 0.69%, 0.34% to 3.37% and 0.22% to 1.76%, respectively. Thus, it has great potential for commercialization due to the high medicinal value (Akhouwah *et al.*, 2004; Chin & Hussin, 2011).

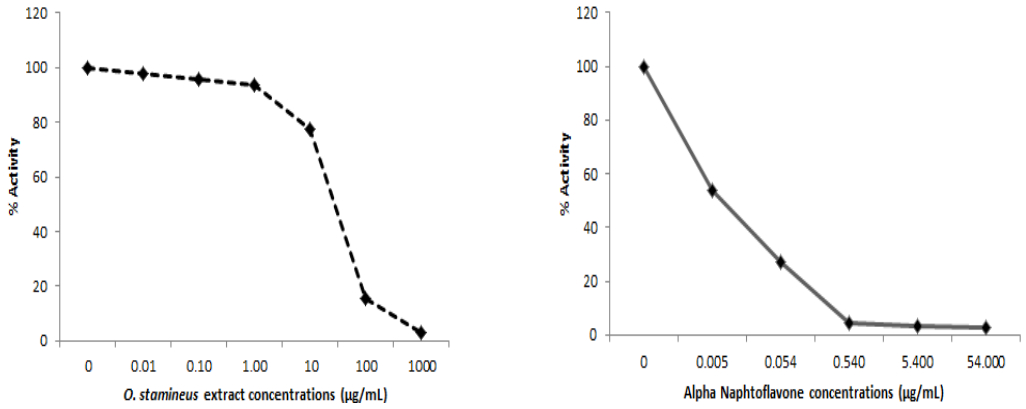

Figure 2. Effect of *O. stamineus* (A) and Alpha Naphtoflavone (B) on the CYP1A2 activity.

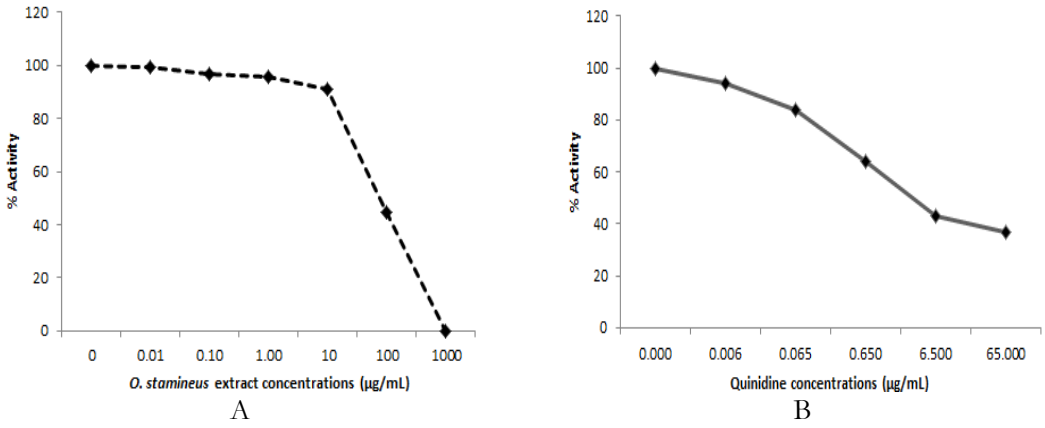

Figure 3. Effect of *O. stamineus* (A) and Quinidine (B) on the CYP2D6 activity

Interactions between herbal remedies and CYP isoforms (CYPs) have been assessed and a number of studies have been published. For example, the inhibitory effects of asiaticoside and madecassoside on human CYPs were studied by Winitthana *et al.* (2011). The results showed that asiaticoside inhibited CYP2C19 and CYP3A4. Madecassoside also inhibited CYP2C19 and CYP3A4, but both asiaticoside and madecassoside had no effect on the activities of CYP1A2, CYP2C9 and CYP2D6 and CYP2E1. Ohnishi *et al.* (2000) reported the effect of furanocoumarin derivatives in grapefruit juice on the activity of cytochrome P450 3A4; dihydroxybergamottin, bergamottin and bergapten showed potent inhibition with IC_{50} ranging from 0.075 to 1.0 µM. In another study, the effects of St John's wort *(Hypericum perforatum)* on human cytochrome P450 activity were investigated by Wang *et al.* (2001); the results indicated that St John's wort was a selective inductor for CYP3A, but did not alter the CYP1A2, CYP2C9 and CYP2D6 activities after St John's wort was administered.

There are a lot of medicinal plants that have been used either in primary forms or combined into mixtures as traditional medicines. Traditional medicine has been assumed to be non-toxic because the compounds originate from natural sources (Zhou *et al.*, 2004). Pharmacologically active constituents such as alkaloids, flavonoids, terpenoids, polyphenols, anthraquinones, glycosides, saponins, coumarins, tannins and essential oils can be found in plants and have the ability to promote herb-drug interactions (Zhou *et al.*, 2004; Markowitz *et al.*, 2000).

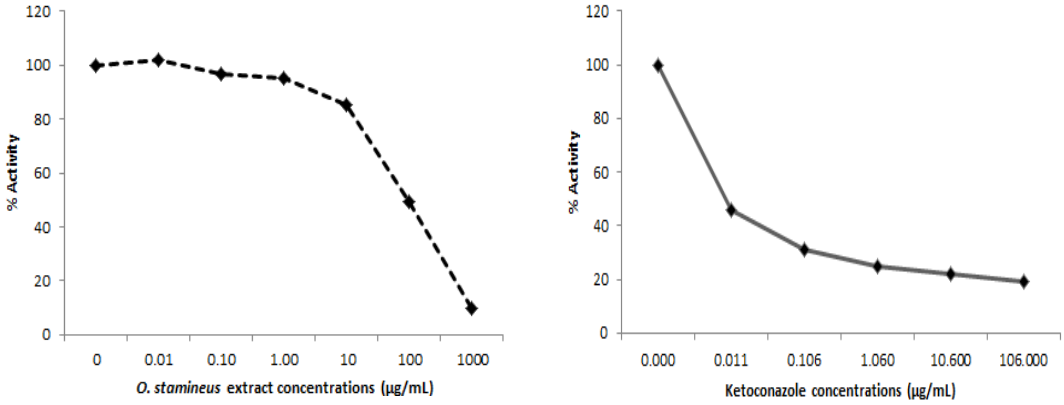

Figure 4. Effect of *O. stamineus* (A) and Ketoconazole (B) on the CYP3A4 activity

Table I. IC_{50} values of O. stamineus extract and positive control after interaction with the four human CYP isoforms

CYP Isoforms	IC_{50} Values	
	Positive control IC_{50}	***O. Stamineus*** Extract IC_{50}
CYP2C9	Sulphaphenazole - 0.03µg/mL	20.12µg/mL
CYP1A2	Alpha Naphtoflavone - 0.007µg/mL	49.90µg/mL
CYP2D6	Quinidine – 2.9µg/mL	89.24µg/mL
CYP3A4	Ketoconazole - 0.003µg/mL	97.82µg/mL

Intestinal PgP and CYP3A4 have an important role in determining many drugs' bioavailability. The modulation of CYP3A and intestinal PgP constitutes an important mechanism of increasing or decreasing the concomitant drug bioavailability (Fugh-Berman, 2000; Fugh-Berman and Ernst, 2001; Izzo and Ernst, 2001). The interaction of *O stamineus* extract with CYP2C9 and CYP1A2 induced moderate inhibition towards the two CYPs (Qiu *et al*., 2008), suggesting that the extract may modulate the metabolism of drugs metabolized by CYP2C9 and CYP1A2. It has been estimated that CYP2C9 is responsible for the metabolic clearance of up to 15-20% of all drugs undergoing phase I metabolism (Booven *et al*., 2010), while the cytochrome *CYP1A2* plays a major role in the metabolism of many drugs that commonly clinical used (5-10%).

CONCLUSION

The standardized extract of *O. stamineus* showed moderate inhibition towards CYP2C9 and CYP1A2 with IC_{50} values of 20.12 µg/mL and 49.90 µg/mL. While interaction study with CYP2D6 and CYP3A4 showed inhibition with IC_{50} values of 89.24 and 97.82 µg/mL, respectively. These data suggested that *O. stamineus* extract may induce herb-drug interactions, since the CYP2C9 or CYP1A2 isoforms are responsible for the metabolism of many drugs.

ACKNOWLEDGEMENT

The authors wish to acknowledge to Prof. Zhari Ismail from School of Pharmaceutical Sciences, Universiti Sains Malaysia who provided the extract for this research project.

REFERENCES

Akowuah GA., Zhari, I., Norhayati I., Sadikun A., Khamsah SM. 2004. Sinensitin, eupatorin, 3'-hydroxy-5, 6, 7, 4'-tetramethoxyflavone and rosmarinic acid contents and antioxidative effect of *Orthosiphon stamineus* from Malaysia. *Food Chem*. 82: 559-566.

Barnes J., Mills SY., Abbot NC., Willoughby M., Ernst E.1998. Different standards

for reporting ADRs to herbal remedies and conventional OTC medicines: face-to-face interviews with 515 users of herbal remedies. *Br. J. Clin. Pharmacol.* 45: 496-500.

Booven DV., Marsh S., McLeod H., Carrillo MW., Sangkuhl K., Klein TE., Altman RB. 2010. Cytochrome P450 2C9-CYP2C9. *Pharmacogenet Genomics*. 20(4): 277-281.

Bosetti A., Sobol M., Ma D., Good T., Liu D., Cali JJ. 2005. Bioluminescent ytochrome P450 assays. *Pharmacology*. 3: 9-18.

Chin JH., Hussin AH., Ismail S. 2009. Effect of *Orthosiphon Stamineus* Leaf Extracts On Hepatic Cytochrome P450, UGT And GST Activity In STZ-Induced Diabetic Rats. *J.Adv. Scie.Arts*. 1(1): 1-8.

Chin JH., Hussin AH. 2011. *Orthosiphon Stamineus* Increases metabolism of Aminopyrine in Female Rat Hepatocytes by Inhibiting Protein Kinase-A. *Int. J. Pharm. Sci. Res*. 2(7): 1777-1780.

Fugh-Berman A. 2000. Herb-drug interactions. *Lancet*. 355: 134-138.

Fugh-Berman A., Ernst E. 2001. Herb-drug interactions: review and assessment of report reliability. *Br. J. Clin. Pharmacol.* 52: 587-595.

Hanapi NA., Azizi J., Ismail S., Mansor SM. 2010. Evaluation of selected Malaysian medicinal plants on phase I drug metabolizing enzymes, CYP2C9, CYP2D6 and CYP3A4 activities *in vitro*. *Int. J. Pharm.* 6(4): 494-499.

Indubala J., Ng LT. 2000. Herbs: The Green Pharmacy of Malaysia 1st ed. Vinpress Sdn. Bhd., Kuala Lumpur, pp.76.

Ioannides C. 2002. Xenobiotic Metabolis: an Overview. In C. Ioannides (Ed.), *Enzymes Systems The Metabolise Drugs and Other Xenobiotics*. John Wiley & Sons, Ltd., West Sussex, pp. 1-30.

Ismail S., Hanapi NA., Halim MRA., Uchaipichat V., Mackenzie PI. 2010. Effects of *Andrographis paniculata* and *Orthosiphon stamineus* extracts on the glucuronidation of 4-methylumbelliferone in human UGT isoforms. *Molecules*. 15: 3578-3592.

Izzo AA., Ernst E. 2001. Interactions between herbal medicines and prescribed drugs: a systematic review. *Drugs*. 61(15): 2163-2175.

Izzo AA., Borrelli F., Capasso R. 2002. Herbal medicine: the dangers of drug interaction. *Trends Pharmacol. Sci.* 23: 358-359.

Markowitz JS, de Vane CL., Boulton DW., Carson SW., Nahaz Z., Risch SC. 2000. Effect of St. John's wort (*Hypericum perforatum*) on cytochrome P-450 2D6 and 3A4 activity in healthy volunteers. *Life Sci*. 66: 33-39.

Ohnishi A., Matsuo H., Yamada S., Takanaga H., Morimoto S., Shoyama Y., Ohtani H., Sawada Y. 2000. Effect of furanocoumarin derivatives in grapefruit juice on the uptake of vinblastine by Caco-2 cells and on the activity of cytochrome P450 3A4. *Br. J. Pharmacol.* 130: 1369-1377.

Pietta P., Simonetti P., Mauri P. 1998. Anioxidant activity of selected medicinal plants. *J. Agric. Food Chem*. 46: 4487–4490.

Qiu F., Zhang R., Sun J., Jiye A., Hao H., Peng Y., Ai H., Wang G. 2008. Inhibitory Effects of Seven Components of Danshen Extract on Catalytic Activity of Cytochrome P450 Enzyme in Human Liver Microsomes. *Drug Metab. Dispos.* 36(7): 1308-1314.

Sumaryono, W., Proksc, P., Wray, V., Witte, L., and Hartmann, T. 1991. Qualitative and quantitative analysis of the phenolic constituents from Orthosiphon aristatus. P*lanta Med.* 57: 176–180.

Tezuka Y., Stampoulis P., Banskota AJ., Awale S., Kadota S., Saiki I. 2000. Constituents of the Vietnamese medicinal plant *Orthosiphon stamineus*. *Chem. Pharm. Bull.* 48: 1711–1719.

Wang Z., Gorski JC., Hamman MA., Huang S-M., Lesko LJ., Hall SD. 2001. The effects of St John's wort *(Hypericum perforatum)* on human cytochrome 450 activity. *Clin. Pharmacol. Ther.* 70: 317-326

Wiart C. 2002. *Orthosiphon stamineus*, Benth. In: F. K. WONG (Ed.). *Medicinal plants of*

Southeast Asia. Prentice Hall, Selangor, pp. 264.

Wilkinson GR. 1997. The effects of diet, aging and disease-states on presystemic elimination and oral drug bioavailability in humans. *Adv. Drug Deliv. Rev.* 27: 129-159.

Winitthana T., Niwattisaiwong N., Patarapanich C., Tantisira MH., Lawanprasert S. 2011. *In vitro* inhibitory effects of asiaticoside and madecassoside on human cytochrome P450. *Toxicology in vitro*. 25 (4): 890-896.

Zhou SF., Gao YH., Wen QJ., Huang M., Xu AL., Paxton JW. 2003. Interactions of herbs with cytochrome P450. *Drug Metab. Rev.* 35: 35-98.

Zhou S., Koh HL., Gao Y., Gong Z., Lee EJD. 2004. Herbal bioactivation: The good, the bad and the ugly. *Life Sci.* 74: 935-968.