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Effects of *Phyllantus Amarus* aqueous extract and *Phyllanthus Emblica* aqueous extract on human CYP2D6 and CYP3A4

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Abstract

Phyllanthus amarus Schum. & Thonn. and *Phyllanthus emblica* Linn. are well-known as herbal medicine with many pharmacological effects. Inhibitory effects of the aqueous extracts of both plants on cytochrome P450 (CYP) including CYP2D6 and CYP3A4, were investigated using recombinant human CYP. The results demonstrated that *P. amarus* aqueous extract and *P. emblica* aqueous extract possessed inhibitory effects on CYP2D6 with IC_{50} of 180.02 μg/mL and 599.34 μg/mL, respectively. Inhibitory effects of *P. amarus* aqueous extract and *P. emblica* aqueous extract on CYP3A4 were shown by the IC_{50} of 2.11 μg/mL and 321.89 μg/mL, respectively. Results from this study provide an information indicating the possibilities of herb-drug interaction if these extracts are co-administered with the prescribing drugs that are metabolizing by CYP2D6 and CYP3A4.

Keywords: *Phyllanthus amarus* , *Phyllanthus emblica*, CYP2D6, CYP3A4, Herb-Drug interaction

Introduction

Herbal medicines have currently become popular as an alternative medicine [1]. Pharmacological activities of several plant species are interesting. *Phyllanthus amarus* Schum. & Thonn. and *Phyllanthus emblica* Linn., the plants in family Euphorbiaceae, have been found in many tropical countries including Thailand. Both herbal-plants are well-known as traditional medicine with various pharmacology activities [2]. Their common activities include antioxidant [3,4], antimicrobial [5,6] as well as hepatoprotective activity from ethanol [7,8] and *N*-nitrosodiethylamine [9] etc. Besides the toxicity data of both plants in animals [10,11], herb-drug interaction investigation is also needed to ensure the safety of using these plants in human. CYP, the phase I enzyme system in the liver, normally plays a key role in metabolism of most currently used medicines. Among CYP isoforms, CYP2D6 and CYP3A4 involve in metabolism of drugs for more than 80% [12]. Modification of CYP has been shown to be one of the most important etiology of drug-drug interaction. Therefore, the aim of the present study was to investigate the inhibition effect of *P. amarus* and *P. emblica* aqueous extracts on CYP2D6 and CYP3A4 by using recombinant human CYPs *in vitro*.

Materials & Methods

The inhibition of the extracts on CYP2D6 and CYP3A4 activities was determined according to the Vivid® CYP450 Screening Kits Protocol (www.invitrogen.com). The principle of this assay was characterized by the inhibition effect of the compound of interest with the specific CYP isoform. Thus, the reaction of changing the Vivid® substrates (7-benzyloxymethyloxy-3-cyanocoumarian; BOMCC or ethyloxymethyloxy-3-cyanocoumarian;

EOMCC) by that specific CYP isoform into a highly fluorescent product was reduced as compared to the reaction without the compound of interest.

The reaction was performed in 96-multiwell black plates. The mixture of the NADPH-regeneration system (comprised Glucose-6-phosphate and Glucose-6-phosphate dehydrogenase in potassium phosphate buffer pH 8.0) and the CYP450 BACULOSOMES® reagent were prepared and added in each wells with 40 μL of the test compound at various concentrations. The plate was pre-incubated for 20 minutes at room temperature. The reaction was started by adding 50 μL of the mixture of $NADP^+$ and the Vivid® substrate solution and incubated for 30-60 min at room temperature. Ten microliters of 0.5 M Tris-base buffer (pH 10.5) was added to stop the reaction. Fluorescence intensity was measured by fluorescence microplate reader ($VICTOR^3$; PerkinElmer, USA) with the excitation wavelength of 405 ± 10 nm and the emission wavelength of 460 ± 20 nm. Validation of the protocol was performed before analyzing the test compounds by using the known selective CYP2D6 or CYP3A4 inhibitors. Miconazole was used as the selective CYP2D6 inhibitor whereas ketoconazole was used as the selective inhibitor of CYP3A4.

Statistical analysis

Percentage of inhibition of each concentration of the test compounds was calculated by using the following equation.

$$\% \text{ Inhibition} = 1 - \left[\frac{(RFU_{\text{test compound}} - RFU_{\text{background of test compound}})}{(RFU_{\text{solvent control}} - RFU_{\text{background of solvent control}})} \right] \times 100$$

Median inhibition concentration (IC_{50}) was calculated by plotting concentrations of the test compound against the corresponding percent inhibition. Data were analyzed using probit analysis of SPSS software version 16.

Results

IC_{50} of miconazole on CYP2D6 was shown to be 0.67 μM whereas the IC_{50} of ketoconazole on CYP3A4 was 0.11 μM (Table 1). The data were consistant to the IC_{50} of both selective inhibitors of the particular CYP reported earlier [13,14].

IC_{50} of *P. amarus* aqueous extract on CYP2D6 and CYP3A4 was shown to be 180.02 μg/mL and 2.11 μg/mL, respectively (Table 1, Figure 1 and 2). While IC_{50} of *P. emblica* aqueous extract on CYP2D6 and CYP3A4 was shown to be 599.34 μg/mL and 321.89 μg/mL, respectively (Table 1, Figure 3 and 4).

Table 1: IC_{50} values of the selective inhibitors, *P. amarus* aqueous extract and *P.emblica* aqueous extract on CYP2D6 and CYP3A4

CYP isoforms	Selective inhibitor	IC_{50} (μM) of selective inhibitor	IC50 (μg/mL) of selective inhibitor	IC_{50} (μg/mL)	
				P. amarus aqueous extract	*P. emblica* aqueous extract
CYP2D6	**miconazole**	**0.67**	**0.32**	**180.02**	**599.34**
CYP3A4	**ketoconazole**	**0.11**	**0.06**	**2.11**	**321.89**

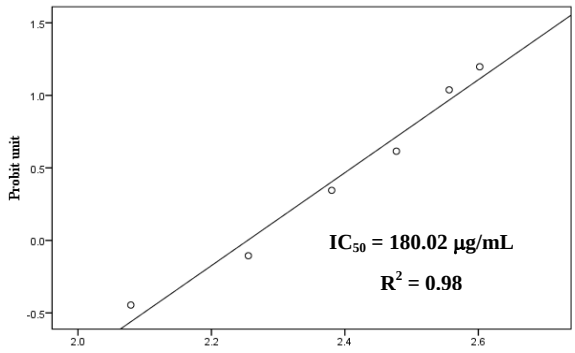

Figure 1: Inhibitory effect of *P. amarus* aqueous extract on CYP2D6. (Each data point represented the mean of triplicate analysis)

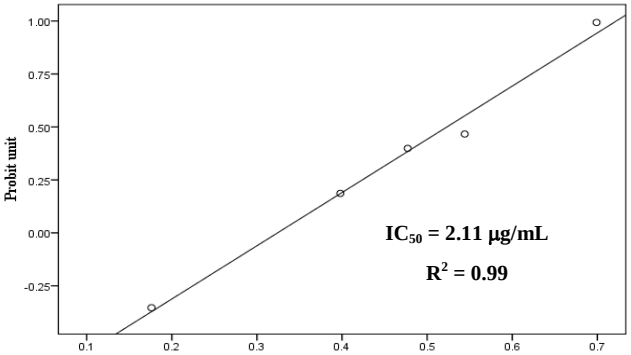

Figure 2: Inhibitory effect of *P. amarus* aqueous extract on CYP3A4. (Each data point represented the mean of triplicate analysis)

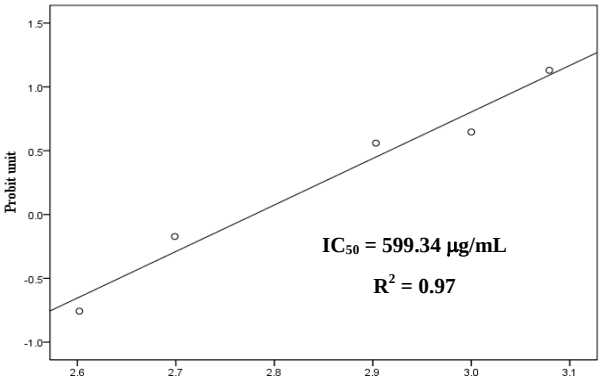

Figure 3: Inhibitory effect of *P. emblica* aqueous extract on CYP2D6. (Each data point represented the mean of triplicate analysis)

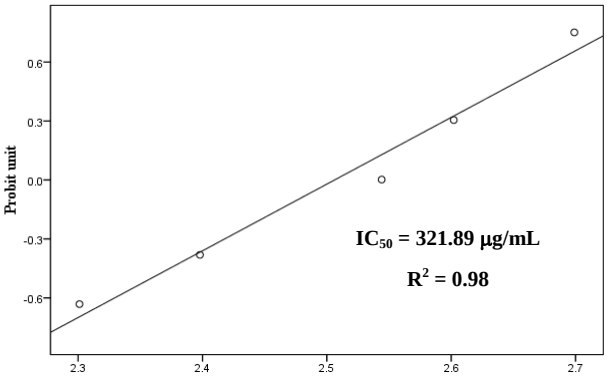

Figure 4: Inhibitory effect of *P. emblica* aqueous extract on CYP3A4. (Each data point represented the mean of triplicate analysis)

References

1. Wilson KM, Klein JD, Sesselberg TS, et al. Use of complementary medicine and dietary supplements among U.S. adolescents. Journal of Adolescent Health. 2006; 38: 385-394.
2. David WU, Grady LW, and Baruch SB. Use and bioassay in *Phyllanthus* (Euphorbiaceae): a complication II. The subgenus *Phyllanthus*. Journal of Ethnopharmacology 1991; 34: 97-133.
3. Lime YY, and Murtijaya. Antioxidant properties of *Phyllanthus amarus* extract as affected by different drying methods. LWT-Food Science and Technology 2007; 40: 1664-9.
4. Luo W, Zhao M, Yang B, et al. Identification of bioactive compounds in *Phyllanthus emblica* L. Fruit and their free radical scavenging activities. Food Chemistry 2009; 114: 499-504.
5. Ahmad I, Mehmood Z, and Mohammad F. Screening of some Indian medical plants for their antimicrobial properties. Journal of Ethnopharmacology 1998; 62: 183-93.
6. Rahman S, Akbor MM, Howlader A, et al. Antimicrobial and cytotoxic activity of the alkaloids of Amlaki (*Emblica officinalis*). Parkistan Journal of Biological Sciences 2009; 12(16): 1152-5.
7. Pramyothin P, Samosorn P, Poungshompoo S, et al. The protective effects of *Phyllanthus emblica* Linn. extract on ethanol induced rat hepatic injury. Journal of Ethnopharmacology 2006; 107: 361-4.

8. Pramyothin P, Ngamtin C, Poungshompoo S, et al. Hepatoprotective activity of *Phyllanthus amarus* Schum. et. Thonn. extract in ethanol treated rats : *In vitro* and *In vivo* studies. Journal of Ethnopharmacology 2007; 14(2): 169 – 73.
9. Jeena KJ, Joy KL, Kuttan R. Effect of *Emblica officinalis*, *Phyllanthus amarus* and *Picrorrhiza kurroa* on *N*-nitrosodiethylamine induced hepatocarcinogenesis. Cancer Letters 1999; 136: 11-6.
10. Evi LP, Kwashie EG, Agbonon A, et al. Toxicological assessment on extracts of *Phyllanthus amarus* Schum. and Thonn. Scientific Research and Essay 2008; 3(9): 410-5.
11. Itthipanichpong C, Ousavaplangchai L, Ramart S, et al. Acute toxicity and subacute toxicity study of *Phyllanthus emblica* L. Chula Med J 1987; 31(5): 367-76.
12. Anzenbacher P, Anzenbacherova E. Review cytochrome P450 and metabolism of xenobiotics. Cellular and Molecular Life Sciences 2001; 58: 737-47.
13. Weaver R, Graham KS, Beattie IG, et al. Cytochrome P450 inhibition using recombinant proteins and mass spectrometry/multiple reaction monitoring technology in a cassette incubation. Drug metabolism and disposition 2003; 31(7): 955-66.
14. Zientek M, Miller H, Smith D, et al. Development of an *in vitro* drug–drug interaction assay to simultaneously monitor five cytochrome P450 isoforms and performance assessment using drug library compounds. Journal of Pharmacological and Toxicological Methods 2008; (58): 206-14.