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

Napaporn Anannarukan¹*, Nuansri Niwattisaiwong², Warangkana Warisnoicharoen², Pornpen Pramyothin¹, Somsong Lawanprasert¹**

¹Department of Pharmacology and Physiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand.
²Department of Food and Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand.
* Presenting Author, ** Corresponding Author

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 IC₅₀ 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 IC₅₀ 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-benzyloxyethoxy-3-cyanocoumarian; BOMCC or ethyloxyethoxy-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 (VICTOR3; 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.

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

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 consistent 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

<table>
<thead>
<tr>
<th>CYP isoforms</th>
<th>Selective inhibitor</th>
<th>IC(_{50}) (μM) of selective inhibitor</th>
<th>IC(_{50}) (μg/mL) of selective inhibitor</th>
<th>IC(_{50}) (μg/mL) P. amarus aqueous extract</th>
<th>IC(_{50}) (μg/mL) P. emblica aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2D6</td>
<td>miconazole</td>
<td>0.67</td>
<td>0.32</td>
<td>180.02</td>
<td>599.34</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>ketoconazole</td>
<td>0.11</td>
<td>0.06</td>
<td>2.11</td>
<td>321.89</td>
</tr>
</tbody>
</table>
Figure 1: Inhibitory effect of *P. amarus* aqueous extract on CYP2D6. (Each data point represented the mean of triplicate analysis)

IC$_{50} = 180.02$ μg/mL  
$R^2 = 0.98$

Log of concentration of *P. amarus* aqueous extract

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

IC$_{50} = 2.11$ μg/mL  
$R^2 = 0.99$

Log of concentration of *P. amarus* aqueous extract

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

IC$_{50} = 599.34$ μg/mL  
$R^2 = 0.97$

Log of concentration of *P. emblica* aqueous extract

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

IC$_{50} = 321.89$ μg/mL  
$R^2 = 0.98$

Log of concentration of *P. emblica* aqueous extract

References


