Inhibitory effects of medicinal folk plants from Ban-Ang-Ed Official Community Forest Project (The Chaipattana Foundation) on drug-metabolizing cytochrome P450 3A4 and 2C9 enzymes

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ABSTRACT

The liver-specific cytochrome P450 3A4 (CYP3A4) and 2C9 (CYP2C9) enzymes are heme containing enzyme that belongs to cytochrome P450 superfamily. Both CYP3A4 and CYP2C9 enzymes are responsible for metabolizing various pharmaceutical drugs (~40-45% of prescribed drugs), suggesting an important role of CYP3A4 and CYP2C9 enzymes in health care treatment. In Thailand, healthcare treatment by co-administration of herbal products along with clinical drugs is becoming popular; however, this situation could lead to various side effects because of herb-drug interaction. In this study we investigate the inhibitory effect of commonly used medicinal folk plants from Ban-Ang-Ed official community forest project (The Chaipattana Foundation) on CYP3A4 and CYP2C9 enzyme. The plants were collected based on local intellectual suggestion of commonly used medicinal folk plants for healthcare treatment. The harvested plants were identified, macerated, and ethanolic extracted. The CYP3A4 and CYP2C9 enzymes were bacterially expressed and purified separately. The inhibitory activity of plant extracts from Ban-Ang-Ed at concentration of 50 µg/ml on CYP3A4 and CYP2C9 enzymatic activities were determined. The results showed that plant extracts differently affected CYP3A4 mediated testosterone 6-β hydroxylation activity and CYP2C9 mediated tolbutamide 4-hydroxylation activity in vitro. Among tested plant extracts, Aglaonema nitidum extract (commonly use for treatment of hemorrhoid) could potently inhibited CYP3A4 and CYP2C9 activities, followed by Picrasma javanica (commonly use for anti-anemia and treatment of inflammation) and Tacca chantrieri (commonly use as elixir and aphrodisiac). According to the results, care must be taken for co-treatment of diseases by using medicinal folk plants in Ban-Ang-Ed with CYP3A4 and CYP2C9 metabolizing pharmaceutical drugs.

Keywords: Cytochrome P450 3A4, Cytochrome P450 2C9, Ban-Ang-Ed Official Community Forest Project, Testosterone 6-β hydroxylation activity, Tolbutamide 4-hydroxylation activity
1. INTRODUCTION

There is a trend nowadays to employ natural products for treatment of diseases. The use of medicinal herbs, either pre-treatment or co-administration with drugs, has particularly increased over the past few years. However, this situation could lead to various side effects because of herb-drug interaction of natural derived compounds with the liver-specific cytochromes P450 3A4 (CYP3A4) and 2C9 (CYP2C9) enzymes that have been reported to metabolized various clinical drugs (~ 40-45% of prescribed drugs). Several reports have demonstrated that natural compounds and herbal products may cause pharmacokinetic interaction with clinically used drugs when co-administrated [1-6]. The Herb–drug interactions (HDIs) due to cytochrome P450 (CYP) induction, inhibition, and/or inactivation can lead to treatment failure, attenuation of the efficacy of concomitant medications and even death in some cases [7-9]. The traditional approach for assessing potential CYP induction or inhibition of test compounds has been to measure the change in enzyme activities of CYP3A4 and CYP2C9 using known CYP-specific probe substrates. In Thailand, many medicinal plants are used as traditionally to cure many diseases and almost unregulated. In addition, many patients do not inform their physician about the traditional medicines they consume. Therefore, interactions between traditional medicines and drugs prescribed clinically are becoming a concern. In this study, some of the selected medicinal plants which are widely used from Ban-Ang-Ed official community forest project (The Chaipattana Foundation) based on local intellectual suggestion were selected and evaluated for their action on CYP3A4 (testosterone 6-β-hydroxylation) and CYP2C9 (tolbutamide 4-hydroxylation) enzymes. The obtained information of HDIs will be beneficial for safety used of these selected plants.

2. MATERIALS AND METHODS

Preparation of herb extracts

The plants were collected based on local intellectual suggestion of commonly used medicinal folk plants for healthcare treatment. The harvested plants have been currently identified by Dr. Benchawon Chewpreecha, Department of Biology, Burapha University and collected in herbarium. All harvested plants were dry, macerate and extract with 95% ethanol for 5 days, filtered and solvent removed by vacuum rotary evaporator.

Cytochrome P450 CYP3A4 and CYP2C9 activity assay

The human CYP3A4, CYP2C9 and rat CPR, a P450s redox partner, proteins were expressed and purified as previously described [10-12], the purified enzymes were then used for P450-reconstitution enzymatic assay. Enzymatic activity of CYP3A4 to metabolize testosterone substrate to 6-β-hydroxytestosterone product (testosterone 6-β-hydroxylation activity) and CYP2C9 to metabolize tolbutamide to 4-hydroxytolbutamide (tolbutamide 4-hydroxylation activity) were determined as previously described [10, 11], with some modification. The purified human CYP3A4 or CYP2C9 enzyme was pre-incubated with rat CPR in 50 mM Tris-Cl buffer for 10 min at room temperature, followed by incubation with 100 µM testosterone substrate for CYP3A4 enzyme and 100 µM tolbutamide substrate for CYP2C9 enzyme. The reaction was further incubated at room temperature for 5 min before starting reaction by addition of NADPH. The reaction was incubated at 37 C for 20 min before addition of 2M HCl (T20 min), addition of androsterdione internal standard and centrifuged for 10 min. The equal volume of Ethyl acetate was added to the supernatant to extract the products followed by centrifugation at 10,000 rpm for 10 min. The Ethyl acetate was removed and re- dissolved in methanol. The products formation was analyzed using a Novapak C18-Reverse Phase High Performance Liquid Chromatography (C18-RP-HPLC) with the mobile phase of methanol: water (80:20, v/v), a flow rate of 0.4 ml/min, and monitoring at 240 nm for 6-β-hydroxytestosterone and 229 nm for 4-hydroxytolbutamide. Total formation of either 6-β-hydroxytestosterone product or 4-hydroxytolbutamide were compared with the initial reaction (T 0 min), addition of 2M HCl right after NADPH addition.

Inhibition of CYP3A4 and CYP2C9 by medicinal folk plants

Inhibition screening assay was carried out by pre-incubation of either purified human CYP3A4 or CYP2C9 enzyme with rat CPR in 50 mM Tris-Cl buffer for 10 min at room temperature, followed by incubation with 100 µM testosterone substrate for CYP3A4 enzyme and 100 µM tolbutamide substrate for CYP2C9 enzyme in the presence of 50 µg/ml of plant extracts for 5 min before starting reaction by addition of NADPH. Products determination was performed as described. The remaining activity of CYP3A4 or CYP2C9 enzymes in the presence of plant extracts were compared with the control reaction in which the DMSO solvent was added instead of tested plant extracts using SPSS and Graph-Pad Prism software, version 5 (La Jolla, CA).
Further study of the inhibitory activity of extract on electron transfer of rat CPR was determined. The purified rat CPR was incubated with cytochrome c in 50 mM Tris-HCL buffer pH 7.5, 50 µM NADPH was add to start reaction, increasing of cytochrome c (reduced form) was detected at 550 nm [12]. Specific activity was analyzed by SPSS and GraphPad Prism5 to verified remaining activity of enzyme compared between with or without extracts.

3. RESULTS

The human CYP3A4, CYP2C9 and rat CPR enzymes were successfully expressed and purified from *E. coli* expression system into homogeneity, as determined by SDS-PAGE (data not shown). The cytochrome c reduction activity of the purified rat CPR is 58.69 ± 1.58 µmol of cytochrome c reduction/minute/mg protein which is comparable to previously report [10]. Using the *in vitro* reconstitution enzymatic assay, the specific activity of purified CYP3A4-mediated testosterone 6β-hydroxylation activity is 0.2887 ± 0.0008 µmol 6β-hydroxytestosterone/minute/mg protein while the specific activity of purified CYP2C9-mediated tolbutamide 4-hydroxylation is 0.2980 ± 0.0016 µmol 4-hydroxytolbutamide/minute/mg protein which are comparable to previously report [8,9].

The present study attempted to screen medicinal folk plant in Ban-Ang-Ed official community forest project (The Chaipattana Foundation) that possessed inhibitory activity against human hepatic CYP3A4 and CYP2C9 enzymes. These selected plants were commonly used for anti-inflammation, anti-flu and antibiotic based on local intellectual suggestion. We found that plant extracts at 50 µg/ml concentrations differently affected both CYP3A4 and CYP2C9 activity *in vitro* (Anova, \( p < 0.05 \)). Interestingly, the enzymatic activity of CYP3A4 was more affected by HDIs compared to the CYP2C9 activity (Figure 1 and 2). The CYP3A4 activity was modulated to the highest activity, almost 3-folds, in the presence of Chongko (leave) and Madihom (leave) extracts followed by Mahunk (root), Hengmaina (trunk), and Klongkleng (leave) extracts. On the other hand, Klongkleng (root), Denguton (leave), and Haadiew (leave) are very potent inhibitory activity against CYP3A4 enzyme followed by kangkaodum (rhizome and leave), Rayomnoi (leave and root) compared to other extracts (Figure 1). In contrast, most of the selected plants merely affected the CYP2C9-mediated tolbutamide 4-hydroxylation activity *in vitro* (Figure 2). There were only Phanomsawan (root) and Uengmaina (rhizome) showed slightly activated CYP2C9 activity while Huadiew (leave), Denguton (leave) showed highest inhibitory activity among other plant extracts.

Figure 1. Effect of selected plant extract on *in vitro* CYP3A4 mediated Testosterone 6β-hydroxylation activity.
As CPR plays role in electrons transfer for P450-mediated metabolism in vitro, and the effect of plant extract on CPR activity could impair metabolic function of various P450 isoforms, resulting in diverse un-predictable side-effects of HDIs. Surprisingly, we found that none of selected extracts (50 µg/ml) affected in vitro cytochrome c reduction activity (data not show), implicated that the modulation in enzymatic activity of CYP3A4 or CYP2C9 enzyme did not through activation or inactivation of CPR enzyme.

According to these results, either pre-treatment or co-administration of medicinal plants should be in caution especially when supplement in patents who under healthcare treatment with CYP3A4 metabolizing drugs such as antibiotic (erythromycin), anti-arrhythmic (quinidine), benzodiazepines (diazepam, midazolam), immune modulators (cyclosporine), HIV antivirals (indinavir, saquinavir), antihistamines (astemizole, chlorpheniramine), calcium channel blockers (amlodipine, felodipine, verapamil), HMG CoA reductase inhibitors (atorvastatin, lovastatin), steroid 6 beta-OH (estradiol, progesterone, testosterone), miscellaneous (caffeine, cocaine, docetaxel, methadone, tamoxifen). For CYP2C9 enzyme, care must be taken in patient who co-administrated Phanomsawan (root), Uengmaina (rhizome), Huadiew (leave) and Denguton (leave) with NSAIDs (diclofenac, ibuprofen, meloxicam) Oral Hypoglycemic Agents (tolbutamide), Angiotensin II Blockers (losartan, irbesartan), Sulfonilureas (glyburide, tolbutamide), Others (amitriptyline, fluvalastatin, tamoxifen S-warfarin) As the inhibition or inactivation could lead to high dose of drugs remaining, so it has side effect for body and failure of drug treatment. In contrast, activation by herbal constituents could lead to rapid elimination of drugs and failure of drug treatment [2, 3, 7]. These HDIs due to cytochrome P450 induction or inhibition, could lead to treatment failure, attenuation of the efficacy of concomitant medications and even death in some cases.

![Figure 2. Inhibitory effect of herbs extract on tolbutamide 4-hydroxylation of CYP2C9.](image)

4. CONCLUSIONS

In this study we investigate the inhibitory effect of commonly used medicinal folk plants from Ban-Ang-Ed official community forest project (The Chaipattana Foundation) on CYP3A4 and CYP2C9 enzyme. The results showed that plant extracts differently affected CYP3A4 mediated testosterone 6-β-hydroxylation activity and CYP2C9 mediated tolbutamide 4-hydroxylation activity in vitro. Among tested plant extracts, Aglaonema nitidum extract (commonly use for treatment of hemorrhoid) could potently inhibited CYP3A4 and CYP2C9 activities, followed by Picrasma javanica (commonly used for antianemia and treatment of inflammation) and Tacca chantrieri (commonly use as elixir and aphrodisiac). According to the results, care must be taken for co-treatment of diseases by using medicinal folk plants in Ban-Ang-Ed with CYP3A4 and CYP2C9 metabolizing pharmaceutical drugs.
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