Inhibitory effects of Thai herbal extracts on the cytochrome P450 3A-mediated metabolism of gefitinib, lapatinib and sorafenib

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Abstract

Herbal products are widely used in cancer patients via co-administration with chemotherapy. Previous studies have demonstrated that pharmacokinetic interactions between herbs and anticancer drugs exist due to inhibition of drug-metabolizing enzymes, particularly cytochrome P450s (CYPs). The aim of this study was to determine the inhibitory effects of Andrographis paniculata, Curcuma zedoaria, Ganoderma lucidum, Murrannia denticulata and Ventilago denticulata extracts on the metabolism of gefitinib, lapatinib and sorafenib. The activities of CYP3A in human liver microsome on the metabolism of gefitinib, lapatinib and sorafenib in the absence and presence of Thai herbal extracts were assayed using high-performance liquid chromatography analysis. Curcuma zedoaria extract potently inhibited CYP3A-mediated lapatinib and sorafenib metabolism with IC₅₀ values of 4.18 ± 3.20 and 7.59 ± 1.23 μg/mL, respectively, while the metabolism of gefitinib was strongly inhibited by Murrannia denticulata and Ventilago denticulata extracts with IC₅₀ values of 7.53 ± 2.87 and 7.06 ± 1.23 μg/mL, respectively. Andrographis paniculata and Ganoderma lucidum extracts had less effect on the metabolism of the tested anticancers (IC₅₀ values >10 μg/mL). In addition, kinetic analysis of the ability of Curcuma zedoaria extract to inhibit CYP3A-mediated metabolism of anticancer drugs was best described by the noncompetitive and competitive inhibition models with Kᵢ values of 20.08 and 11.55 μg/mL for the metabolism of gefitinib and sorafenib, respectively. The present study demonstrated that there were potential pharmacokinetic interactions between tyrosine kinase inhibitors and herbal extracts.

1. Introduction

Targeted therapy is an emerging issue in cancer treatment. Tyrosine kinase inhibitors (TKIs) are a group of drugs that inhibit the tyrosine kinase domain of epidermal growth factor receptor (EGFR) and other targets, resulting in disruption of the signal transduction pathways of protein kinase. Inhibition of downstream signal transduction pathways of tyrosine kinases can modify cell growth, migration, differentiation, apoptosis and death [1]. Gefitinib is an active, selective ATP-competitive inhibitor of the tyrosine kinase domain on the EGFR target. It has been approved by the Food and Drug Administration (FDA) for first-line treatment of advanced non-small cell lung cancer [2]. After oral administration, gefitinib is metabolized mainly by cytochrome P450 (CYP) 3A4 in the liver while catalysis by CYP1A1, CYP3A5 and CYP2D6 play minor roles [3,4]. The metabolites of gefitinib metabolism by CYP3A4/5 are 3-deisopropylnyl-3-hydroxyethylamino gefitinib.

Abbreviations: ATP, adenosine 5’-triphosphate; CYP, cytochrome P450; DMSO, dimethyl sulfoxide; EGFR, epidermal growth factor receptor; FDA, Food and Drug Administration; G–6–P, glucose–6–phosphate; G–6–PD, G–6–P dehydrogenase; GEF, gefitinib; HER2, human epidermal growth factor receptor 2; HLM, human liver microsome; HPLC, high-performance liquid chromatography; IC₅₀, half maximal inhibitory concentration; Kᵢ, inhibition constant; LC/MS, liquid mass spectrometry; NADP, nicotinamide adenine dinucleotide phosphate; PDGFR, platelet-derived growth factor receptor; RAF, Rapidly Accelerated Fibrosarcoma; SOR, sorafenib; TKI, tyrosine kinase inhibitor; UGT, UDP-glucuronosyltransferase; UV, ultraviolet; VEGFR, vascular endothelial growth factor receptor.

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and 4-defluoro-4-hydroxy gefitinib. The formation of O-desmethyl–gefitinib, a major metabolite in human plasma, is mediated by CYP2D6 [4]. Lapatinib is the first dual-selective ATP–competitive inhibitor used for the treatment of advanced breast cancer [5,6]. It targets two members of tyrosine kinase receptors including EGFR and human epidermal growth factor receptor 2 (HER2) [7]. Following oral administration, lapatinib is metabolized by CYP3A4, CYP3A5, CYP2C8 and CYP2C19 in which CYP3A4 is the major enzyme [8]. Lapatinib is normally metabolized into O-dealkylated, N-dealkylated and N-hydroxylated metabolites [9]. In addition, sorafenib is a multitargeted inhibitor drug with targeting vascular endothelial growth factor receptors (VEGFRs), platelet–derived growth factor receptors (PDGFRs) and RAF kinases [10,11]. It is approved for the treatment of advanced renal cell carcinoma, hepatocellular carcinoma and follicular thyroid carcinoma [5,6]. Sorafenib is mostly metabolized in the liver by two pathways including sorafenin N-oxidation by CYP3A4 and sorafenin glucuronidation by UGT1A9 [12-14].

However, in Thailand, a large proportion of cancer patients also have the tendency to use alternative medicines, particularly herbal therapy, either alone or in combination with conventional therapies. The reasons why cancer patients choose to use traditional treatments are that they want to reduce the side effects of the treatment drugs and to enhance their general well-being. However, co–administration of herbal therapy with anticancer drugs can result in adverse effects by induction or inhibition of drug–metabolizing enzymes, particularly the CYPs. The inhibition of CYP activities could delay the elimination of anticancer drugs and lead to drug accumulation, while the induction of CYP enzymes can lead to increased metabolic activity and reduced drug exposure. The potential for herb–drug interactions is high when TKIs are co–administered with CYP3A4 inhibitors (herbal extracts) due to the interruption of the normal process of TKI metabolism by CYP3A4 enzymes. This fact increases the risk of drug toxicity in cancer patients who co-administer TKIs with herbal medicines. Thus, the objective of this study was to determine the inhibitory effects of Thai traditional herb extracts including Andrographis paniculata (Burm.f.) Nees (A. paniculata), Curcuma zedoaria (Christm.) Roscoe (C. zedoaria), Ganoderma lucidum (G. lucidum), Murrannia loriiformis (Hassk.) R.S.Rao & Kammathy (M. loriiformis) and Ventilago denticulata Willd. (V. denticulata) on the CYP3A–mediated metabolism of gefitinib, lapatinib and sorafenib.

2. Materials and methods

2.1. Herbs

Herbs including A. paniculata, C. zedoaria, G. lucidum, M. loriiformis and V. denticulata were purchased from Vejpongpharmacy, a local herb distributor (Bangkok, Thailand), as dried herbal products.

2.2. Chemicals and reagents

Gefitinib, lapatinib and sorafenib were purchased from LC Laboratories (Woburn, MA, USA). Glucose—6-phosphate (G—6—P), G—6—P dehydrogenase (G—6—PD), nicotinamide adenine dinucleotide phosphate (NADP), testosterone and ketoconazole were purchased from Sigma–Aldrich (St. Louis, MO, USA). Acetonitrile, ammonium acetate, acetic acid, dimethyl sulfoxide (DMSO) and methanol were purchased from Merck KGaA (Darmstadt, Germany). High purity water was from MilliQ Synergy UV Ultrapure water system (Merck KGaA, Darmstadt, Germany). All other solvents and reagents were analytical grade or higher. Pooled human liver microsomes (HLMs; from 50 donors) were purchased from Gibco BRL Life Technologies (Grand Island, NY, USA).

2.3. Preparation of herbal extracts

Twenty–five g of commercial dried herbal product was dissolved in 125 mL of 99.9% methanol with gently shaking at room temperature for 4 h on a rotary shaker. After maceration, herbal solution was sonicated in an ultrasonic water bath at room temperature for 10 min. The homogenized suspension was filtered twice through Whatman® no. 1 filter paper and evaporated to dryness by a rotary evaporator and a speed vacuum concentrator. All the extracts were kept at –20 °C until use.

2.4. Determination of major constituents in herbal extract

Previous published HPLC and LC/MS-MS methods were modified to determine the major constituents in A. paniculata, C. zedoaria, G. lucidum, M. loriiformis and V. denticulata (Supplement data). Herbal extract was dissolved in methanol to form 0.5–5 mg/mL solution. The solution was sonicated for 10 min, then filtered with Syringe Filter 0.45 μm Nylon. Each sample solution was analyzed by HPLC or LC/MS-MS.

2.5. Inhibitory effects of herbal extracts toward the metabolism of gefitinib, lapatinib and sorafenib

To investigate whether herbal extracts inhibited CYP3A-mediated metabolism of TKIs, experiments were performed using the concentrations of herbal extracts and TKIs plus their incubation times as listed in Table 1. Initially, 100 μL of reaction contained HLMs (0.5 mg/mL), phosphate buffer (0.1 M; pH 7.4), TKIs and herbal extract or DMSO (negative control). Herbal extracts were dissolved in DMSO to form 10 mg/mL solutions, where final concentration of organic solvent did not exceed 2% (v/v). Ketoconazole was used as a selective CYP3A inhibitor at the concentration range of 0.005 – 5 μM. Concentrations of the substrate in reaction mixtures were chosen near the Michaelis–Menten (Km) value for CYP3A4 activity in HLMs. Pre–incubation was performed at 37 °C for 5 min in a shaking water bath and then NADPH-generating system (1 mM NADP, 10 mM G—6—P, 2 IU G—6—PD and 5 mM MgCl2) was added. After incubation, the reaction mixtures were terminated by adding 100 μL of ice–cold 4% acetic acid in methanol. Reaction mixtures were then vortex–mixed, cooled on ice for 10 min and centrifuged (5000 g for 10 min at 4 °C). The supernatant was aliquoted and injected into the HPLC. The incubation time was determined to ensure that the reaction velocity was in the linear range.

2.6. Inhibitory effects of herbal extracts toward 6β-testosterone hydroxylation

To investigate the inhibitory effects of herbal extracts on the activity of CYP3A, testosterone conversion to 6β–hydroxytestosterone was performed to evaluate CYP3A enzyme activity. The incubation of HLMs with testosterone was performed in the same way as described above except HLMs (0.25 mg/mL) were incubated with 40 μM of testosterone for 15 min and the reactions terminated with 1 μL of 70% perchloric acid. Reaction mixtures were then vortex–mixed, cooled on ice for 10 min and centrifuged (5000 g for 10 min at 4 °C). The supernatant was aliquoted and injected into the HPLC.

2.7. Inhibition kinetics of C. zedoaria extract on the metabolism of gefitinib and sorafenib

To determine the enzyme inhibition types (competitive, noncompetitive, uncompetitive or mixed–type inhibition), different

| Drug          | Concentration of drug (μM) | Concentration of herb (μg/mL) | Incubation time (min) |
|---------------|-----------------------------|-------------------------------|-----------------------|
| Gefitinib     | 40                          | 2.5 – 1000                    | 30                    |
| Lapatinib     | 2.5                         | 0.5 – 250                     | 15                    |
| Sorafenib     | 2.5                         | 2.5 – 500                     | 30                    |
| Testosterone  | 40                          | 0.5 – 500                     | 15                    |
concentrations of gefitinib (20, 40 and 80 μM), sorafenib (10, 20 and 40 μM) and C. zedoaria (0, 2.5, 5, 10 and 20 μg/mL) were incubated with HLMs. Incubation conditions were performed as described above. The concentrations of gefitinib, sorafenib and their metabolites were quantified by HPLC.

2.8. Measurement of gefitinib, lapatinib, sorafenib and their metabolites by HPLC

The concentrations of gefitinib, lapatinib, sorafenib and their metabolites were determined by following previously published HPLC methods with few modifications [3,15]. The HPLC system was an Agilent 1200 series instrument (Agilent, Germany). The UV detection was set at the suitable wavelength for each analyte (Table 2). Analytes were separated on Ascentis® C18 analytical column [150 mm × 4.6 mm (id), 5 μm; Supelco, USA] using composition of mobile phases and running time as shown in Table 2. The mobile phase flow rate was 1.0 mL/min. The standard curve was constructed over the concentration ranges of 0 – 40 μM.

2.9. Measurement of testosterone by HPLC

The HPLC method used to analyze testosterone 6β−hydroxylation was modified from Polasek et al. [16]. Testosterone and 6β−hydroxysterosterone were separated on Ascentis® C18 analytical column [150 mm × 4.6 mm (id), 5 μm; Supelco, USA]. The mobile phases were 5% acetonitrile in water (mobile phase A) and acetonitrile (mobile phase B) starting with 25% of mobile phase B over 5 min. Mobile phase B was changed to 50% over 6 min, then to 90% over 1 min which was held for 30 s before returning to the initial condition. UV detection was set at 241 nm. The standard curve was constructed over the concentration ranges of 0 – 40 μM. The retention time of testosterone was 10.9 min

2.10. Data analysis

The inhibitory effects of herbal extract on the metabolism of gefitinib, lapatinib and sorafenib were performed in quadruplicate. The concentrations of gefitinib, lapatinib, sorafenib, 3−desmethyl−3−hydroxyethylamino gefitinib, sorafenib N−oxide and testosterone were calculated from the integrated peaks in the HPLC chromatograms compared to each substrate’s standard curve. The half maximal inhibitory concentration (IC50) values were determined from the logarithmic plot of herbal extract concentration versus the percentage of remaining enzyme activity using GraphPad Prism software (version 8.2.1(279); GraphPad Software, California, USA). The inhibition constant (K) values were determined by EnzFitter (version 2.0, Biosoft, UK). The model equation used to calculate IC50 value from the plot was:

\[
\% \text{ inhibition} = 100(1+10^{\log IC})
\]

where A is a logarithm of the concentration of herbal extract in the incubation.

Table 2

| Drug          | Mobile phase | Detector wavelength (nm) | Retention time (min) | Reference |
|---------------|--------------|--------------------------|----------------------|-----------|
| Gefitinib     | 60% A, 40% C| 250                      | 9.2                  | [3]       |
| Lapatinib     | 50% B, 50% C| 262                      | 8.4                  | [15]      |
| Sorafenib     | 50% B, 50% C| 265                      | 9.7                  | [15]      |

A: 0.4% ammonium acetate, B: 10 mM ammonium acetate (pH 5.7) containing 10% acetonitrile, C: acetonitrile

3. Results

3.1. Determination of major constituents in herbal extract

In this study, major constituents in the extract of A. paniculata, C. zedoaria, G. lucidum, M. loriformis and V. denticulata were identified by HPLC and LC-MS/MS methods. The chromatograms and detected phytochemical compounds were provided in the Supplementary data. The andrographolide content in A. paniculata extract was found to be 19.6%. The curcuminoids (curcumin, demethoxycurcumin and bisde-methoxycurcumin) content in C. zedoaria extract was found to be 1.8%. The triterpenoids compounds in G. lucidum detected by LC-MS/MS could be classified into ganoderic acids, lucidic acids and ganoderenic acids according to their chemical structures and MS fragmentation patterns. Extract of M. loriformis contained isovitexin and β-D-glucopyranosyl-2-(2'-hydroxy-Z-6'-enecosamide) sphingosine. Several compounds in V. denticulata extract were identified as listed in Table S3. Among them, emodin, physcion and ventilagodenin A were previously isolated and reported by Molee et al. [17].

3.2. Inhibition of CYP3A−catalyzed metabolism of gefitinib, lapatinib, sorafenib and testosterone by herbal extracts

CYP3A isoenzyme is the most abundant CYP enzyme in the human liver, and it is the primary enzyme responsible for the metabolism of gefitinib, lapatinib and sorafenib. Ketoconazole is a specific inhibitor of CYP3A activity, so in this study it was used as a positive control. As an inhibitor of CYP3A catalytic activity, ketoconazole potently inhibited the metabolism of gefitinib, lapatinib and sorafenib with IC50 Values of 0.03, 0.18 and 0.06 μg/mL, respectively. These data confirmed the role of CYP3A activity in HLMs regarding metabolism of gefitinib, lapatinib and sorafenib.

To evaluate herb−drug interactions of five Thai herbal extracts, including A. paniculata, C. zedoaria, G. lucidum, M. loriformis and V. denticulata, herbal extracts were co−incubated with gefitinib, lapatinib and sorafenib and testosterone. Testosterone conversion to 6β−hydroxysterosterone is a commonly used assay to evaluate CYP3A enzyme activity. Moderate to potent inhibition was observed with the herbal extracts on CYP3A−mediated testosterone 6β−hydroxylation with a relative order of inhibitory potency as follows: C. zedoaria (IC50 1.05 ± 0.36 μg/mL) > G. lucidum (IC50 7.45 ± 6.64 μg/mL) > V. denticulata (IC50 27.22 ± 3.82 μg/mL) > M. loriformis (IC50 46.66 ± 9.99 μg/mL) > A. paniculata (IC50 77.69 ± 10.43 μg/mL). As per the IC50 values listed in Table 3, C. zedoaria extract was the most potent inhibitor of lapatinib and sorafenib (IC50 < 10 μg/mL), and it moderately inhibited gefitinib metabolism. V. denticulata and M. loriformis strongly inhibited the metabolism of gefitinib with IC50 values of 7.06 ± 1.23 and 7.53 ± 2.87 μg/mL, respectively. On the other hand, relatively weak inhibitions (IC50 > 60 μg/mL) were observed for A. paniculata (with lapatinib and sorafenib) and G. lucidum (with gefitinib and sorafenib). Furthermore, at higher concentration of each herbal extract (250, 500 or 1000 μg/mL), they appeared to almost completely inhibit the activity of CYP3A in regards to metabolism of gefitinib, lapatinib and sorafenib as the half maximal inhibitory concentration (IC50) (μg/mL).

Table 3

| Thai herb extract | The half maximal inhibitory concentration (IC50) (μg/mL) |
|------------------|---------------------------------------------------------|
|                  | Gefitinib | Lapatinib | Sorafenib |
| A. paniculata    | 16.09 ± 7.74 | 117.90 ± 27.84 | 59.04 ± 0.35 |
| C. zedoaria      | 12.17 ± 3.44 | 4.18 ± 3.20  | 7.59 ± 1.23  |
| G. lucidum       | 76.21 ± 30.67 | 11.10 ± 3.27  | 149.10 ± 1.04 |
| M. loriformis    | 7.53 ± 2.87  | 56.81 ± 17.65 | 28.45 ± 8.91 |
| V. denticulata   | 7.06 ± 1.23  | 11.69 ± 2.64  | 47.42 ± 5.03  |

Data were means ± S.D. of quadruplicate determination.
shown in Figs. 1–3.

3.3. Inhibition kinetics of C. zedoaria extract toward the metabolism of gefitinib and sorafenib

To further elucidate the mechanism of inhibition by C. zedoaria extract on CYP3A activity, inhibition kinetics of gefitinib and sorafenib were performed in the HLM system. Note that kinetic analysis of lapatinib was not determined due to undetectability of its metabolite. Kinetic analyses showed that the inhibitory effect of gefitinib 3–desmorpholinyl–3-hydroxyethylamino oxidation and sorafenib N–oxidation by C. zedoaria extracts displayed noncompetitive and competitive inhibition properties with Ki values of 20.08 and 11.55 µg/mL, respectively. The Dixon plots are shown in Fig. 4.

4. Discussion

A large majority of TKIs is metabolized, at least to a certain extent, by CYP3A4/5 [18]. Ketoconazole is known as a selective CYP3A4/5 inhibitor and the FDA recommends using ketoconazole as a strong CYP3A4/5 inhibitor for the study of drug–drug interactions both in vitro and in vivo [19]. Tseng et al. [20] showed that among 32 drugs known to be predominantly metabolized by CYP3A, only 7 drugs were highly metabolized by CYP3A5 whereas 17 of them showed negligible CYP3A5 contribution [20]. In addition, the inhibitory potency of ketoconazole in recombinant CYP3A5 activity seemed to be less than in recombinant CYP3A4 and HLMs [21]. Therefore, CYP3A5 is probably less important in herb–drug interactions. In our study, the metabolism of gefitinib, lapatinib and sorafenib were strongly inhibited by ketoconazole with IC50 values of 0.06, 0.34 and 0.12 µM, respectively, indicating that CYP3A exhibits significant catalytic activity towards the metabolism of gefitinib, lapatinib and sorafenib. In the present study, the inhibitory effects of methanol herbal extracts, including A. paniculata, C. zedoaria, G. lucidum, M. lorumis and V. denticulata on the metabolism of gefitinib, lapatinib and sorafenib catalyzed by CYP3A in HLMs were evaluated. As testosterone 6β–hydroxylation is a common mode of CYP3A activity testing, all herbal extracts moderately to potently inhibited testosterone metabolism with IC50 ranging from 1.05 to 77.69 µg/mL. Our findings revealed that C. zedoaria, M. lorumis and V. denticulata extracts moderately to potently inhibited the metabolism of tested TKIs, while A. paniculata extract appeared to moderately inhibit gefitinib metabolism and G. lucidum extract strongly inhibited the metabolism of lapatinib.

The pattern of CYP3A4 activity inhibition was substrate–dependent, similar to previous reports. For instance, ginsenosides, the major active component of ginseng, inhibited CYP3A4 via a substrate–dependent phenomenon when using HLMs and different substrates [22-24]. This phenomenon might be explained by the relatively large active site cavity and the conformational flexibility of CYP3A4 [25]. Based on a study of CYP3A4 peptides, they contain at least two or three distinct ligand binding sites, in which a substrate and an inhibitor can bind to the enzyme simultaneously albeit with different affinities [25].

The main active components of the rhizome of C. zedoaria were the nonvolatile curcuminoids (curcumin, demethoxycurcumin and bisde-methoxycurcumin) and volatile oils (sesquiterpenoids and monoterpenoids) [26-28]. A study of CYP3A4 activity in Caco–2 cells found that C. zedoaria extract (0.1 mg/mL) decreased 6β–hydroxytestosterone and oxidized nifedipine formation by 98% and 85%, respectively [29]. Another study showed that curcumin inhibited the activities of recombinant CYP3A4 toward the metabolism of benzoxylresorufin and dibenzylfluorescein with IC50 values of 16.3 and 13.9 µM, respectively. In addition, the inhibition kinetics were best described by a competitive inhibition model (Ki 7.4 µM) [30]. Volak et al. [31] demonstrated that the effects on CYP3A activities toward triazolam 1′–hydroxylation were similar for the individual curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin) when compared to a curcuminoid mixture and a curcuminoid extract with IC50 values ranging from 32.1 to 58.0 µM. In addition, the inhibition of CYP3A4 by the curcuminoid extract fit well with a competitive inhibition model (Ki 11.0 µM) [31]. Our data showed that C. zedoaria extract noncompetitively and competitively inhibited the metabolism of gefitinib and sorafenib with Ki values of 20.08 and 11.55 µg/mL, respectively.

M. lorumis and V. denticulata are widely cultivated throughout Thailand. M. lorumis is known as Ya Pak King in Thai, and it is used to treat leukemia and cancer [32]. V. denticulata is also called as Tao Wan Lek or Rang Dang in Thai. Its antioxidant and cytotoxic activities were found in its aqueous and ethanolic extracts [33]. A phytochemical study showed that M. lorumis contains glycosphingolipids and phytosteryl...
Numerous substances containing an anthraquinone group (e.g., chrysophenol, physcion and emodin) as well as naphthalene derivatives (ventilagolin, ventilagodenin A and ventilagodenin B) were isolated from *V. denticulata* [17,35]. Despite the fact that they are commonly found and used in Thailand, information regarding their inhibition of drug metabolizing enzymes is limited. In vitro testing showed that emodin strongly inhibited testosterone 6β-hydroxylation (IC₅₀ 9.6 μM) and moderately affected midazolam 4-hydroxylation (IC₅₀ 37.3 μM) [36]. In the present study, both *M. loriformis* and *V. denticulata* extracts strongly inhibited CYP3A-mediated gefitinib metabolism (IC₅₀ < 10 μg/mL).

**Fig. 2.** Inhibitory effects of Thai traditional herbal extracts on CYP3A-mediated lapatinib metabolism (A) *A. paniculata*, (B) *C. zedoaria*, (C) *G. lucidum*, (D) *M. loriformis* and (E) *V. denticulata*. Each data point represents mean ± S.D. of quadruplicate determination.

Even the methanol extracts of *A. paniculata* and *G. lucidum* seemed to be less potent when inhibiting CYP3A activities, and they showed moderate inhibitory effects on the metabolism of gefitinib (IC₅₀ 16.09 μg/mL for *A. paniculata*) and lapatinib (IC₅₀ 11.10 μg/mL for *G. lucidum*). *A. paniculata* is known in Thai as Fah Tha Lai Jone. Its major constituents that provide biological properties are andrographolide, 11,12-didehydro-14-deoxyandrographolide and neoandrographolide [37,38]. The methanol extract of *A. paniculata* and andrographolide have been shown to inhibit the activities of human recombinant CYP3A4 on testosterone 6β-hydroxylation (IC₅₀ 27.8 and 86.1 μg/mL, respectively) [39]. Studies by Pekthong et al. [40,41] revealed that crude...
extract of A. paniculata competitively inhibited CYP3A4–dependent testosterone 6β–hydroxylation in HLMs with an IC50 value of 34.1 μM. Also, when they exposed human hepatocytes to either andrographolide (50 μM) or A. paniculata extract (containing 50 μM andrographolide), the inhibitory effect of the extract was more potent [40,41].

G. lucidum is commonly known as Ling Zhi in Chinese, and it has been used as a traditional Chinese medicine to promote health and longevity [42]. Polysaccharides, peptidoglycans and triterpene are three major active constituents in G. lucidum, and their biological activities have been reviewed elsewhere [43,42,44]. Xu et al. [45] reported that ganoderic acid A, one of the most abundant triterpenoids of G. lucidum extract, noncompetitively inhibits the activity of CYP3A4 on testosterone metabolism with an IC50 value of 15.05 and 7.16 μM, respectively [45]. Another study showed that G. lucidum polysaccharide dose–dependently inhibit the activity of CYP3A–catalyzed nifedipine oxidation in HLMs with an IC50 value of 783 μg/mL [46]. According to a previous study, the concentrations of ganoderic acid in plasma ranged from 2.57 to 10.99 ng/mL after oral administration of 3000 mg Ling Zhi [47]. These concentrations were much less than the IC50 values found in the inhibition studies. Therefore, the inhibitory effect of ganoderic acids may be very weak.

Actually, the in vitro inhibition cannot represent that the herbal extracts will cause clinically relevant interactions. Currently available human pharmacokinetic data show a low exposure of bioactive compounds even at very high doses. Plasma concentrations of andrographolide, curcuminooids and ganoderic acids in human were found to be in nanomolar ranges [48,49,47]. The inhibition parameters determined in the present study, including IC50 and KI values, are in micromolar ranges which are relatively high compared to the anticipated amounts of bioactive compounds in the liver. Although, there are no reports on the pharmacokinetic studies of M. loriformis and V. denticulata.

In conclusion, this study demonstrated that Thai herbal extracts including A. paniculata, C. zedoaria, G. lucidum, M. loriformis and V. denticulata can decrease TKI metabolism activities, particularly gefitinib, lapatinib and sorafenib in HLMs. It has to be noted that herbal extracts are mixtures of various constituents. The bioactive compounds, contents and concentrations can vary substantially. Further studies are needed to confirm the clinical relevance of the inhibitory effects of herbal extracts on CYP3A enzymes in vivo.

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**CRediT authorship contribution statement**

Chumphorn Rodseeda: Methodology, Validation, Formal analysis, Investigation, Writing – original draft. Paveena Yamanont: Methodology, Validation, Investigation, Writing – review & editing. Darawan Pinthong: Conceptualization, Validation, Resources, Writing – review & editing, Supervision. Porntipa Korpaserthaworn: Conceptualization, Methodology, Validation, Resources, Writing – review & editing, Funding acquisition.

**Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Data Availability**

Data will be made available on request.

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**Appendix A. Supporting information**

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.toxrep.2022.10.004](https://doi.org/10.1016/j.toxrep.2022.10.004).

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