INHIBITORY EFFECTS OF ACTIVE CONSTITUENTS AND EXTRACTS OF ANDROGRAPHIS PANICULATA ON UGT1A1, UGT1A4, AND UGT2B7 ENZYME ACTIVITIES

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ABSTRACT

Objective: The present investigation addresses the inhibitory potential of five Andrographis paniculata (AP) extracts of different polarity and its three active constituents on UGT1A1, UGT1A4 and UGT2B7.

Methods: Bioluminescent assay with luciferin as a substrate was used to determine IC50 values for all extracts and constituents. The kinetic enzyme inhibition experiments were subsequently performed to determine Ki values and inhibition modes for extracts and constituents having IC50 values less than 10 μg/mL.

Results: Our results in general exhibit that AP extracts and constituents potently inhibited UGT1A1 and UGT2B7 with varying degrees of inhibition featuring Ki values from 1.0 up to 7.5 μg/mL. On the other hand, none of them showed significant inhibitory effect on UGT1A4. Of the extracts tested, AP ethanolic, AP aqueous (root) and AP aqueous (leaf) were found to inhibit UGT1A1, while the remaining, were devoid of any potent interaction. In contrast, AP ethanolic was found to exclusively and competitively inhibit UGT2B7. Of the constituents examined, andrographolide and 14-Deoxy-11,12-didehydroandrographolide were found to inhibit the activity of UGT2B7, while neo andrographolide and 14-Deoxy-11,12-didehydroandrographolide inhibited UGT1A1, partially implying their relative content in the extracts, consequently representing correlation with inhibition seen in the corresponding extracts.

Conclusion: These findings suggest that AP extracts could cause herb-drug interactions via inhibition of UGT iso forms. An in vivo study is needed to examine this further.

Keywords: Andrographis paniculata; UDP-Glucuronosyltransferase; Glucuronidation; Herb-drug interactions; Bioluminescent assay.

INTRODUCTION

Herbal-drug interactions are generally characterized as either pharmacodynamic, via the site of action at the drug-receptor level, or pharmacokinetic, involving absorption, distribution, metabolism and excretion [1]. The most commonly documented interactions are pharmacokinetic interactions secondary to inhibition of drug metabolism [2]. UDP-glucuronosyl transferase (UGT) is a superfamily of drug metabolizing enzymes that mediate the glucuronidation of various endogenous (e.g., bilirubin, steroid hormones, bile acids, etc.) and exogenous compounds including drugs and phytochemicals, in general rendering them biologically inactive [3]. Several reports document the clinical significance of drug-drug interactions through UGT enzymes [4]. Since many phytochemicals are substrates for UGT enzymes such as flavonoids [5], luteolin, quercetin [6], and curcumin [7], herb-drug interactions may occur through the interference of UGT enzymes [8].

Andrographis paniculata (Hempedu bumi) is a traditional medicinal plant that has been used in Asia for the treatment of fever, flu, asthma, sore throat, bronchitis, flatulence, dysentery, malaria, diabetes and high blood pressure [9]. Andrographolide, neoandrographolide and 14-Deoxy-11,12-didehydroandrographolide (Figure 1) are the diterpenoids, reputed to be among the major therapeutic properties attributed to this plant [10]. Literature survey have shown a wide spectrum of pharmacological importance of the extracts of A. paniculata and its active constituents including hepatoprotective, hypoglycaemic, cardioprotective, anti-inflammatory, immune stimulatory, anti-cancer, anti-HIV and antimalaria [11-14].

Fig. 1: Structures of three A. paniculata active constituents: (A) andrographolide, (B) neoandrographolide, and (C) 14-Deoxy-11,12-didehydroandrographolide.
Andrographolide, neoandrographolide and 14-Deoxy-11,12-didehydroandrographolide (root, stem and leaf) and its three main active constituents andrographolide, neoandrographolide and 14-Deoxy-11,12-didehydroandrographolide may be responsible for some herb-drug interactions caused by combination of A. paniculata-derived medicines and other drugs. In our laboratory, the inhibitory effects of A. paniculata metabolizing enzyme extract on human UGT enzymes activities were previously investigated [23]. To gain additional insight into its interference on glucuronidation, we further examined the inhibitory effects of five A. paniculata extracts of different polarity (ethanol, methanol, aqueous (root, stem and leaf)) and its three main active constituents (andrographolide, neoandrographolide and 14-Deoxy-11,12-didehydroandrographolide) on human UGT1A1, UGT1A4 and UGT2B7 activities as these enzymes are most commonly listed in catalyzing drug glucuronidation [24].

MATERIALS AND METHODS

Materials

Diclofenac sodium, trifluoroperazine dihydrochloride, andrographolide (purity ≥ 98%), neo andrographolide (purity ≥ 95%) and 14-Deoxy-11,12-didehydroandrographolide (purity ≥ 95%) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). All other laboratory chemicals used were of the highest commercially available quality. The luminescent UGT enzyme assay kits (UGT-GoTM) were obtained from Promega Corporation (Madison, WI). The kits contained luminogenic UGT enzyme substrates (a UGT multienzyme substrate or a UGT1A4 selective substrate), UGT buffer, UDPGA solution, D-Cysteine solution, reconstitution buffer, luciferin detection reagent, control microsomes devoid of UGT activity and microsomes containing recombinant human UGT enzymes (Supersomes). All UGT microsomes were included in the kit except UGT1A4 was purchased from BD Biosciences, Discovery Labware (Bedford, MA). White opaque 96-well luminometer microplates were purchased from Greiner Bio-One (Germany).

Preparation of Andrographis paniculata (AP) extracts

Preparation of AP ethanolic extract

Dried and powdered aerial parts of Andrographis paniculata (1 kg) was extracted with 95% ethanol at 60°C in a Soxhlet extractor. The ethanol extract, after concentrated, (160 g of the ethanol extract are obtained from 1 kg starting material) was then analysed by HPLC. The extract was dissolved in distilled water to prepare a 5 mg/mL stock solution [23].

Preparation of AP methanolic extract

The 50:50 (ethanol:water) extract provided by Nova Laboratoires Sdn. Bhd, Selangor, Malaysia were further extracted in Soxhlet apparatus with methanol to make it as methanolic extract. The extract was then filtered through Whatman No.1 filter paper. The solvent of filtrate was dried by evaporation under vacuum into dry powder form.

Preparation of AP aqueous extracts

Fresh whole plant of AP collected from Herbal Garden, School of Pharmaceutical Sciences, Universiti Sains Malaysia was separated into 3 parts; leaf, stem and root. The separated parts were dried and ground. The ground plant parts were applied respectively to reflux into 3 parts; leaf, stem and root. The separated parts were dried and filtered through Whatman No.1 filter paper. The solvent of filtrate was added. The extract was finally obtained by methanol to make it as methanolic extract. The methanol of the extract was evaporated under vacuum to obtain 5 mg/mL stock solution [23].

Preparation of Andrographis paniculata (AP) extracts

HPLC analysis was performed using an Agilent Technologies series 1100 Compact LC system/series equipped with EZChrom Elite software. Compounds were well separated on a 5 μm, 4.6 x 150 mm
RESULTS
A total of five AP extracts of different polarity (ethanol, methanol, aqueous (root, stem and leaf)) and its three main active constituents (andrographolide, neandrographolide and 14-Deoxy-11,12-didehydroandrographolide) were assessed in vitro for their inhibitory effects on the activities of human UGT1A1, UGT1A4 and UGT2B7. Results from the screening experiments are summarized in Table 1. IC50 values were determined based on remaining enzyme activity data at each concentration of test inhibitors. AP extracts and active constituents that showed inhibition of UGT enzyme with IC50 values less than 10 μg/mL were studied further in kinetic assays to determine Ki (inhibition constant) values and inhibition modes.

Inhibitory effects of AP extracts and active constituents on human UGT1A1, UGT1A4 and UGT2B7 enzymes activities

Table 1: IC50 values of positive inhibitors, AP extracts and active constituents on human UGT1A1, UGT1A4 and UGT2B7 enzymes activities

<table>
<thead>
<tr>
<th>Inhibitors*</th>
<th>IC50 (μg/mL)</th>
<th>UGT1A1</th>
<th>UGT1A4</th>
<th>UGT2B7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diclofenac</td>
<td>25.2 ± 17</td>
<td></td>
<td>10.4 ± 1.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(79.1 ± 5.4 μM)</td>
<td></td>
<td>(32.6 ± 3.8 μM)</td>
<td></td>
</tr>
<tr>
<td>Trifluoroperazine</td>
<td>-</td>
<td>18.4 ± 0.03</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(384 ± 0.1 μM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>APEE</td>
<td>5.9 ± 0.2</td>
<td>159.9 ± 8.7</td>
<td>4.5 ± 2.2</td>
<td></td>
</tr>
<tr>
<td>APME</td>
<td>27.4 ± 7.9</td>
<td>128.7 ± 4.7</td>
<td>25.5 ± 1.6</td>
<td></td>
</tr>
<tr>
<td>APAE (root)</td>
<td>3.5 ± 0.3</td>
<td>&gt;1000</td>
<td>111 ± 22.1</td>
<td></td>
</tr>
<tr>
<td>APAE (stem)</td>
<td>30.8 ± 7.0</td>
<td>&gt;1000</td>
<td>20.5 ± 1.7</td>
<td></td>
</tr>
<tr>
<td>APAE (leaf)</td>
<td>6.3 ± 3.1</td>
<td>&gt;1000</td>
<td>16.6 ± 4.7</td>
<td></td>
</tr>
<tr>
<td>AND</td>
<td>&gt;35 (100 μM)</td>
<td>&gt;35 (100 μM)</td>
<td>0.6 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>NeoAND</td>
<td>0.7 ± 0.04</td>
<td>&gt;48 (100 μM)</td>
<td>&gt;48 (100 μM)</td>
<td></td>
</tr>
<tr>
<td>ddAND</td>
<td>2.3 ± 1.0</td>
<td>&gt;33.2 (100 μM)</td>
<td>1.7 ± 0.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(6.9 ± 3.1 μM)</td>
<td></td>
<td>(5.2 ± 0.9 μM)</td>
<td></td>
</tr>
</tbody>
</table>

* AP: Andrographis paniculata, APEE: AP ethanolic, APME: AP methanolic, APAE: AP aqueous, AND: andrographolide, NeoAND: neandrographolide, ddAND: 14-Deoxy-11,12-didehydroandrographolide, ** Data represent best-fit IC50 ± standard error of two determinations. Inhibitors that did not reach 50% inhibition by the highest tested concentration are denoted as IC50 values above the highest tested concentration

Inhibitory effects of AP extracts and active constituents on UGT1A1

AP extracts and active constituents greatly inhibited UGT1A1 with different potencies (Table 1). IC50 of 5.9, 3.5, 6.3, 0.7 and 2.3 μg/mL for AP ethanolic extract, AP aqueous (root) extract, AP aqueous (leaf) extract, neandrographolide and 14-Deoxy-11,12-didehydroandrographolide respectively were further determined for Ki values to characterize the kinetics of UGT1A1 enzyme inhibition. The mechanism of inhibition by the extracts and constituents is of the mixed-type, with exclusive of 14-Deoxy-11,12-didehydroandrographolide as shown by the Lineweaver-Burk plot in Figure 2 (E), which revealed common intercepts at the ordinate consistent with competitive inhibition. Using secondary plots of the slopes (Figure 3), estimates of the Ki were 6.2, 1.7, 7.5, 1.6 and 1.6 μg/mL respectively (Table 2).

Inhibitory effects of AP extracts and active constituents on UGT1A4

As shown in Table 1, AP extracts and active constituents did not significantly inhibit UGT1A4 as the IC50 values were more than the highest tested concentration (1000 μg/mL for extracts and 100 μM for constituents), excluding AP ethanolic extract and AP methanolic extract showing respective weak inhibition with IC50 of 15.9 μg/mL and 128.7 μg/mL. No subsequent kinetic determinations were done as IC50 values were more than 10 μg/mL.

Table 2: Ki values and inhibition modes of AP extracts and active constituents on human UGT1A1, UGT1A4 and UGT2B7 enzymes activities

<table>
<thead>
<tr>
<th>Inhibitors*</th>
<th>Ki (μg/mL)</th>
<th>UGT1A1</th>
<th>UGT1A4</th>
<th>UGT2B7</th>
</tr>
</thead>
<tbody>
<tr>
<td>APEE</td>
<td>6.2 (MIX)**</td>
<td>n.d</td>
<td>2.9 (Com)</td>
<td></td>
</tr>
<tr>
<td>APME</td>
<td>n.d</td>
<td>n.d</td>
<td>n.d</td>
<td></td>
</tr>
<tr>
<td>APAE (root)</td>
<td>1.7 (MIX)</td>
<td>n.d</td>
<td>n.d</td>
<td></td>
</tr>
<tr>
<td>APAE (stem)</td>
<td>n.d</td>
<td>n.d</td>
<td>n.d</td>
<td></td>
</tr>
<tr>
<td>APAE (leaf)</td>
<td>7.5 (MIX)</td>
<td>n.d</td>
<td>n.d</td>
<td></td>
</tr>
<tr>
<td>AND</td>
<td>n.d</td>
<td>1.0 (2.8 μM) (NC)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NeoAND</td>
<td>1.6 (3.3 μM) (MIX)</td>
<td>n.d</td>
<td>n.d</td>
<td></td>
</tr>
<tr>
<td>ddAND</td>
<td>1.8 (5.5 μM) (Com)</td>
<td>1.8 (5.5 μM) (NC)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* AP: Andrographis paniculata, APEE: AP ethanolic, APME: AP methanolic, APAE: AP aqueous, AND: andrographolide, NeoAND: neandrographolide, ddAND: 14-Deoxy-11,12-didehydroandrographolide, ** Ki values determined from secondary plots of the slopes of Lineweaver-Burk plots versus inhibitor concentrations, *** Mode of inhibition: Com - competitive; Mix - mixed-type; NC - non competitive, n.d: not determined as IC50 was more than 10 μg/mL

Inhibitory effects of positive inhibitors on UGTs

To verify the selectivity and inhibitory properties of our assay on the UGT enzymes, the known UGT inhibitors were incubated in the reaction mixture. Diclofenac was used for UGT1A1 and UGT2B7 and trifluoroperazine for UGT1A4. The IC50 values were then compared with those in the literature. High inhibitory potentials were observed for both inhibitors (Table 1). Diclofenac decreased UGT1A1 and UGT2B7 activities with IC50 of 7.91 μM and 32.6 μM respectively, in accordance with the values previously reported [26], and IC50 of 38.4 μM for trifluoroperazine, was close to the previous reported value [27]. These results confirm previous findings on the utility of a luminogenic UGT assay in screening for potential UGT inhibitors using human cDNA-expressed UGT enzymes.
Fig. 2: Lineweaver–Burk plots of human UGT1A1 inhibition by (A) AP ethanol extract, (B) AP aqueous extract (root), (C) AP aqueous extract (leaf), (D) neoandrographolide, and (E) 14-Deoxy-11,12-didehydroandrographolide. Each data point represents the mean of triplicate determinations.
Fig. 3: Secondary plots of the slopes (from Lineweaver-Burk plots of UGT1A1 inhibition) vs. the concentrations of (A) AP ethanol extract, (B) AP aqueous extract (root), (C) AP aqueous extract (leaf), (D) neoandrographolide, and (E) 14-Deoxy-11,12-didehydroandrographolide. Each data point represents the mean of triplicate determinations.
Inhibitory effects of AP extracts and active constituents on UGT2B7

UGT2B7 activities were variably inhibited by the extracts and constituents, exhibiting potent to mild degrees of inhibition, with IC₅₀ values from 0.6 to 111 μg/mL. For neo andrographolide, no significant inhibition was seen as the compound did not reach 50% inhibition by the highest concentration tested (Table 1). AP ethanolic extract, andrographolide and 14-Deoxy-11,12-didehydroandrographolide were selected for further kinetic study. The corresponding Kᵢ values were 2.9, 1.0 and 1.8 μg/mL in competitive and non-competitive modes for the two of the latter respectively (Table 2 and Figure 4).

Determination of active constituents content in AP extracts

The analyses of active constituents in AP extracts were carried out using HPLC methods. Standard curves constructed for andrographolide neoandrographolide and 14-Deoxy-11,12-didehydroandrographolide were linear between 0.001 – 5 ppm, 0.1 – 10 ppm and 0.01 – 20 ppm with the respective coefficient (r²) of 1, 0.9998 and 0.9999 (data not shown). The contents of constituents in AP extracts were listed in Table 3.

DISCUSSION

In this study, the effects of AP extracts and its three constituents on the glucuronidation activities of recombinant human UGT1A1, UGT1A4 and UGT2B7, were examined using a bioluminescent assay to evaluate the possibility of herb-drug interactions due to the inhibition of UGTs. The reliability of this assay was previously confirmed by Larson et al., [26], as close correlations of IC₅₀ values in their work with literature. In addition, our results on the positive inhibitors as described above (in result section), were in agreement with their result, again ascertain the findings.
demonstrated to be involved in the glucuronidation of structurally related terpenoid compounds, in addition to the remarkably lower metabolism by UGT1A4 reported in the studies [28, 29]. The compounds, artemisinin and farnesol are sesquiterpenes originally discovered and isolated from Artemisia annua and many aromatic plants, respectively, have received considerable attention due to its apparent antimalarial and anti cancer properties correspondingly. Among the three constituents, andrographolide showed the highest inhibitory effect on UGT2B7, consistent in it being the highest compound contained in AP ethanolic extract (Table 3), in which the extract exhibited the most potent inhibition on UGT2B7. A pharmacokinetic study had shown that steady-state plasma concentration of andrographolide in human was approximately 0.66 μg/mL (1.9 μM) following ingestion of AP extract at therapeutic dose regime, 3 x 4 tablets/day, about 1 mg/kg body weight/day [30]. This concentration is about one order of magnitude less than the observed andrographolide Ki value for UGT2B7 inhibition, suggesting that inhibition of UGT2B7-mediated systemic glucuronidation by andrographolide seems unlikely. However, based on the estimated intestinal fluid volume of 0.5 to 5.0 L [31], andrographolide concentrations are expected to fall in the range of 12 to 120 μg/mL following consumption of AP extract containing 60 mg (3 x 20 mg) andrographolide, suggesting that the inhibition of andrographolide on first pass metabolism of UGT2B7 substrates is possible. Thus, andrographolide should be used carefully with the drugs metabolized by UGT2B7 such as diclofenac, ephedrine, morphine, codeine, zidovudine [32-34], in order to avoid drug interactions.

Table 3: Content of active constituents in AP ethanol, methanol and aqueous (root, stem and leaf) extract

<table>
<thead>
<tr>
<th>AP extract *</th>
<th>Content of active constituents (%) **</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AND ***</td>
</tr>
<tr>
<td>AP ethanol</td>
<td>1.60</td>
</tr>
<tr>
<td>AP methanol</td>
<td>1.59</td>
</tr>
<tr>
<td>AP aqueous (root)</td>
<td>0.026</td>
</tr>
<tr>
<td>AP aqueous (stem)</td>
<td>0.40</td>
</tr>
<tr>
<td>AP aqueous (leaf)</td>
<td>0.046</td>
</tr>
</tbody>
</table>

* AP: Andrographis paniculata. ** Mean of three determinations in which the results varied by 15% or less., ***AND: andrographolide, NeoAND: neoandrographolide, ddAND: 14-Deoxy-1,12-didehydroandrographolide, nd: not detectable under the assay condition.

To place into perspective the magnitude of UGT1A1 inhibition observed in AP ethanolic extract, as it seems to be the main extract contributing towards this isoform inhibition, a total plasma concentration of AP ethanolic extract was estimated based on previously mentioned published data [30]. Since quantification of total steady-state plasma concentration of all constituents of AP extract is a demanding work, andrographolide is considered as a representable reference compound for all AP extract constituents. Hence, a total plasma concentration of AP extract of about 13.2 μg/mL can be estimated at a recommended dosing of AP extract containing 5% andrographolide, which is higher than our Ki value of 2.9 μg/mL, suggesting that andrographolide may be mainly attributed to the UGT2B7 inhibition by AP ethanolic. On the basis of 14-Deoxy-1,12-didehydroandrographolide (ddAND) low Ki value on UGT2B7 and its undetectable content in AP ethanolic extract (it seems to have no correlation between both parameters), ddAND exhibit no significant contribution at all on this AP ethanolic effect. However, when the effect was considered individually as a compound, ddAND may cause moderate inhibition on UGT2B7, as judged from its Ki value that fall within the range of 1-10 μM [35]. Since no pharmacokinetic data are available on ddAND, a relevant in vitro-in vivo comparison could not be made. Further pharmacokinetic study of ddAND in human is needed to clarify its propriety to interact with UGT2B7 substrate. The assessment of UGT1A1 inhibition by AP extracts demonstrates potent interaction in the order of decreasing effect; AP aqueous (root) > AP ethanolic > AP aqueous (leaf) as depicted by their respective Ki values. Using similar estimate as that made for UGT2B7, all the three AP extracts display Ki values in physiologically reachable concentration based on the estimated total plasma concentration of the extracts, which was higher than their Ki values, suggesting inhibition of glucuronidation of UGT1A1 substrate may be possible. Until more clinical data are available, it seems difficult to clearly determine whether AP extracts have clinically important effects or not on drugs, such as raloxifene and ezetimibe, that are cleared mainly by intestinal first pass UGT1A1 [36, 37].

Characterization of the magnitude of UGT1A1 inhibition by AP constituents reveals that neoandrographolide and ddAND significantly inhibit UGT1A1 with no notable effect from andrographolide. In general, neoandrographolide seems to have some contributions to the inhibition seen in the extracts, mainly AP ethanolic and AP aqueous (leaf) extracts, representing its relatively high content in both extracts. For ddAND, it seems no strong correlation exists between its total content and Ki value as ddAND was not detectable in the extracts in which potent inhibition was seen (i.e. AP ethanolic, AP aqueous (root) and AP aqueous (leaf)), implying that other active constituents in the extracts are suspected to contribute to some extent to this effect, which may include other diterpene lactones, flavonoids, quinic acid derivatives as well as stigmasterols and xanthones. This underestimation might interestingly explain the occurrence of synergistic or antagonistic effects in phytomedicines when more than one inhibitory agent is present [38]. Of note, the potency of UGT1A1 inhibition by AP methanolic and AP aqueous (stem) extracts is expected to cause only weak clinical interactions with drugs metabolized by this isoform, in accordance with the non parallel correlation between its relatively high andrographolide and ddAND content with their fairly moderate IC50 values (27.4 and 30.8 μg/mL respectively). Taken together, however, there is a major gap regarding inhibitory in vitro-in vivo extrapolation, as no study presently exists concerning pharmacokinetic of neoandrographolide and ddAND in human, hence, no relevance correlation could be made between the in vitro inhibition data with known in vivo neoandrographolide and ddAND plasma concentrations. Clinical trials to evaluate the potential risk of interaction of both diterpene along with other constituents on UGTs remain to be conducted.

The evaluation of UGT1A4 inhibition demonstrated that neither AP extracts nor active constituents showed significant inhibitory effects on this isoform. On the basis of this observation, we do not expect that AP extracts and its constituents will serve as either a victim or perpetrator of clinically relevant drug interactions involving drugs metabolized by UGT1A4 such as tacrolimus [39], lamotrigine [40], tamoxifen [41], midazolam [42], and azole antifungals [43]. However, subsequent clinical studies in human subject are needed to confirm the effect.

Although some enzyme inhibition can result in fatal drug interactions, there are circumstances which may result in clinically beneficial interactions. An example of this is the co-medication of valproic acid, a UGT2B7 inhibitor, with another anticonvulsant, lamotrigine, which is primarily eliminated by glucuronidation. This interaction is known to increase the plasma concentration of lamotrigine, allowing the dose of the latter to be reduced, hence, avoiding the side-effect of cutaneous skin rash associated with toxicity [44]. The same applies to herb-drug interactions, as noted recently by a reviewer [45], black pepper and its main constituent piperine may be nominated as one of the most promising...
bioavailability enhancer to improve exposure to certain rapidly metabolized agent. Therefore, in light of increased use of *Andrographis paniculata* in the current preclinical testing as a combination therapy especially for antimalaria and upper respiratory tract infections [46, 47, 48], it is vital to predict appropriately the clinical significance of potential pharmacokinetic drug/herb interactions during the combination therapy.

CONCLUSION

In summary, a bioluminescent assay, using luciferin as a marker substrate, has been validated to investigate the potential inhibition of five AP extracts and three active constituents on UGT1A1, UGT1A4, and UGT2B7 activities. On the basis of our data, AP ethanolic extract, andrographolide and ddAND potently inhibited UGT2B7 while the remaining showed negligible effects. UGT1A1 was inhibited by AP ethanolic extract, AP aqueous (root) extract, AP aqueous (leaf) extract, neoaandrographolide and ddAND. Apparently, no remarkable effect observed from UGT1A4. Until further information surrounding the in vivo pharmacokinetics of these AP and its constituents is obtained, it is suggested that co-medication of such AP preparations with other clinically prescribed drugs should be taken with caution especially that are primarily cleared by the same pathway.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

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