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Original Article

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 IC_{50} values for all extracts and constituents. The kinetic enzyme inhibition experiments were subsequently performed to determine K*i* values and inhibition modes for extracts and constituents having IC_{50} 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 K*i* values from 1.0 upto 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, neoandro grapholide 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 hepato protective, hypoglycaemic, cardioprotective, anti-inflammatory, immune stimulatory, anti-cancer, anti-HIV and antimalaria [11-14].

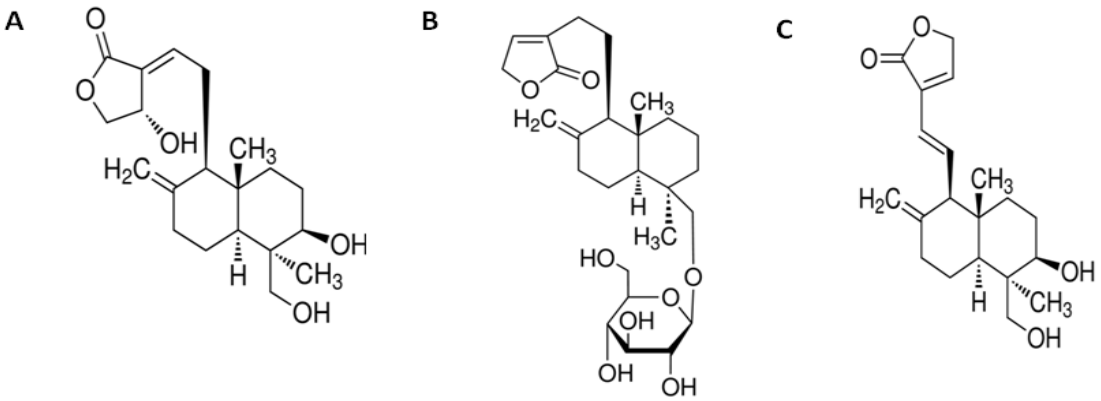

Fig. 1: Structures of three *A. paniculata* active constituents: (A) andrographolide, (B) neoandrographolide, and (C) 14-Deoxy-11,12-didehydroandrographolide.

A. paniculata have been reported to modulate certain drug metabolizing enzymes such as CYP2C9, CYP2D6, CYP3A4 [15-17], CYP2C11 [18], CYP2C/2E1-dependent aniline hydroxylase [19], superoxide dismutase, glutathione S-transferase [20], CYP1A1-dependent ethoxyresorufin *O*-dealkylase and CYP2B10-pentoxyresorufin *O*-dealkylase [21]. Cui et al., [22] have characterized seven metabolites of andrographolide as glucuronide conjugates in human urine. Therefore, it is surmised that glucuronidation 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* ethanolic 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-GloTM) 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 Laboratories 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 apparatus with distilled water for 16 hours. The extracts were then filtered through Whatman No.1 filter paper. The filtrates were reduced by evaporation under vacuum until completely dry. The yield of the extracts (dry form) in terms of their raw material weights were 14.16, 12.1 and 10.5% (w/w) respectively.

Quantification of andrographolide, neoandrographolide and 14-Deoxy-11,12 di-dehydro andrographolide content in *Andrographis paniculata* extracts

HPLC analysis was performed using an Agilent Technologies series 1120 Compact LC system/series equipped with EZChrom Elite software. Compounds were well separated on a 5 µM, 4.6 x 150 mm, Zorbax Eclipse XDB-C18 column using acetonitrile: water (30:70, v/v) at a flow rate of 1 ml/min and at a wavelength of 230 nm. The total run-time was set to 30 min. The retention times of andrographolide, neoandrographolide and 14-Deoxy-11,12-didehydroandrographolide were 5.1, 12.9 and 23.2 min respectively.

AP extracts (ethanolic, methanolic and aqueous) were dissolved in methanol to form 10 mg/mL solutions. The solutions were further diluted to 50 ppm and then centrifuged at 3000 rpm for 1 min. The solutions were injected onto HPLC in volumes of 20 µL to obtain andrographolide, neo andrographolide and 14-Deoxy-11,12-didehydroandrographolide peak area, which were used according to the calibration curve to quantify the content of the three active constituents in each AP extract. The percentage of these compounds by weight was calculated as follows:

$$\% \text{ of compound} = \frac{\text{calculated weight of compound (µg)}}{\text{total dry weight of injected extract (µg)}} \times 100$$

Preparation of stock and working solutions of positive inhibitors, herbal extracts and active constituents

Diclofenac and trifluoroperazine were prepared in distilled water to obtain 100 mM stock solution. All extracts were dissolved in distilled water to achieve a maximum stock solution of 40 mg/mL. The exceptions were AP ethanolic extract and AP methanolic extract, which were prepared at 100 mg/mL in 100% ethanol and methanol respectively. 15 mM andrographolide, 15 mM neo andrographolide, and 50 mM 14-Deoxy-11,12-didehydroandrographolide stock were made by dissolving in 100% methanol. Working solutions were prepared such that the concentrations were 4-fold higher than that required for the final concentrations in incubations by serially dilution with distilled water. Final organic solvents concentrations in incubations were <1%, which did not interfere with the experiment (data not shown). All solutions were freshly prepared at the time of the assays.

UGT inhibition experiments

The positive inhibitors, extracts and active constituents were tested in preliminary screening to determine IC_{50} values by using a single concentration of enzyme and substrate that had been optimized by manufacturer [25]. For each experiment, negative and positive control incubations were performed. Control micro somes instead of UGT micro somes were used in negative control reaction. Positive control was the UGT reaction in the absence of inhibitor. All reaction incubations were carried out in white opaque 96-well lumino meter microplates using a 37°C incubator. Briefly, the final incubation mixture (40 µL per well) consisted of different concentration of each test inhibitors (0.01 – 1000 µM positive inhibitor or 0.1 – 1000 µg/mL extracts or 0.01 – 100 µM active constituents), 4 mM UDPGA or an equivalent volume of water, UGT reaction buffer (8 mM $MgCl_2$, 50 mM TES, pH 7.5), 0.1 mg/mL UGT1A1 or UGT2B7 with 20 µM UGT multienzyme substrate or 0.2 mg/mL UGT1A4 microsomes with 50 µM UGT1A4 substrate. The reactions were terminated by adding 40 µL reconstituted luciferin detection reagent plus D-Cysteine after 60, 90, or 120 min for UGT2B7, UGT1A1 or UGT1A4 respectively to generate a stable glow-type luminescent signal, in which were read directly by microplate reader in luminescence mode after 20 min incubation at room temperature.

AP extracts and active constituents with IC_{50} values less than 10 µg/mL were subsequently determined for K*i* values and inhibition modes. Experiments were carried out by incubating a range of substrate concentration around its Km with a range of inhibitor concentration around its IC_{50} in the presence of UGT microsomes.

Data analysis

IC_{50} (concentration of inhibitor causing inhibition of enzyme activity by half) values were calculated by non-linear regression curve fit using Graph Pad Prism version 5.01 (San Diego, CA, USA). The modes of inhibition were decided graphically from primary Line weaver-Burk plots. K*i* (dissociation constant of the enzyme-inhibitor complex) values were later determined *via* secondary plots of the slopes of Line weaver-Burk plots *versus* inhibitor concentrations.

RESULTS

A total of five AP 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) were assessed *in vitro* for their inhibitory effects on the activities of isolated human UGT1A1, UGT1A4 and UGT2B7. Results from the screening experiments are summarized in Table 1. IC_{50} 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 IC_{50} values less than 10 µg/mL were studied further in kinetic assays to determine K*i* (inhibition constant) values and inhibition modes.

Inhibitory effects of positive inhibitors on UGTs

To verify the selectivity and inhibitive 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 IC_{50} values were then compared with those in the literature. High inhibitive potentials were observed for both inhibitors (Table 1). Diclofenac decreased UGT1A1 and UGT2B7 activities with IC_{50} of 79.1 µM and 32.6 µM respectively, in accordance with the values previously reported [26], and IC_{50} 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.

Table 1: IC_{50} values of positive inhibitors, AP extracts and active constituents on human UGT1A1, UGT1A4 and UGT2B7 enzymes activities

Inhibitors*	IC_{50} (µg/mL)**		
	UGT1A1	UGT1A4	UGT2B7
Diclofenac	25.2 ± 1.7 (79.1 ± 5.4 µM)	-	10.4 ± 1.2 (32.6 ± 3.8 µM)
Trifluoroperazine	-	18.4 ± 0.03 (38.4 ± 0.1 µM)	-
APEE	5.9 ± 6.2	159.9 ± 8.7	4.5 ± 2.2
APME	27.4 ± 7.9	128.7 ± 4.7	25.5 ± 1.6
APAE (root)	3.5 ± 0.3	>1000	111 ± 22.1
APAE (stem)	30.8 ± 7.0	>1000	20.5 ± 1.7
APAE (leaf)	6.3 ± 3.1	>1000	16.6 ± 4.7
AND	>35 (100 µM)	>35 (100 µM)	0.6 ± 0.1 (1.7 ± 0.3 µM)
NeoAND	0.7 ± 0.04 (1.5 ± 0.1 µM)	>48 (100 µM)	>48 (100 µM)
ddAND	2.3 ± 1.0 (6.9 ± 3.1 µM)	>33.2 (100 µM)	1.7 ± 0.3 (5.2 ± 0.9 µM)

* AP: *Andrographis paniculata*, APEE: AP ethanolic, APME: AP methanolic, APAE: AP aqueous, AND: andrographolide, NeoAND: neoandrographolide, ddAND: 14-Deoxy-11,12-didehydroandrographolide, ** Data represent best-fit IC_{50} ± standard error of two determinations. Inhibitors that did not reach 50% inhibition by the highest tested concentration are denoted as IC_{50} 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). IC_{50} 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, neoandrographolide and 14-Deoxy-11,12-didehydroandrographolide respectively were further determined for K*i* 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 K*i* were 6.2, 1.7, 7.5, 1.6 and 1.8 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 IC_{50} 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 IC_{50} of 159.9 µg/mL and 128.7 µg/mL. No subsequent kinetic determinations were done as IC_{50} values were more than 10 µg/mL.

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

Inhibitors*	Ki (µg/mL)**		
	UGT1A1	UGT1A4	UGT2B7
APEE	6.2 (Mix)***	n.d	2.9 (Com)
APME	n.d	n.d	n.d
APAE (root)	1.7 (Mix)	n.d	n.d
APAE (stem)	n.d	n.d	n.d
APAE (leaf)	7.5 (Mix)	n.d	n.d
AND	n.d	n.d	1.0 (2.8 µM) (NC)
NeoAND	1.6 (3.3 µM) (Mix)	n.d	n.d
ddAND	1.8 (5.5 µM) (Com)	n.d	1.8 (5.5 µM) (NC)

* AP: *Andrographis paniculata*, APEE: AP ethanolic, APME: AP methanolic, APAE: AP aqueous, AND: andrographolide, NeoAND: neoandrographolide, ddAND: 14-Deoxy-11,12-didehydroandrographolide., ** K*i* 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 IC_{50} was more than 10 µg/Ml

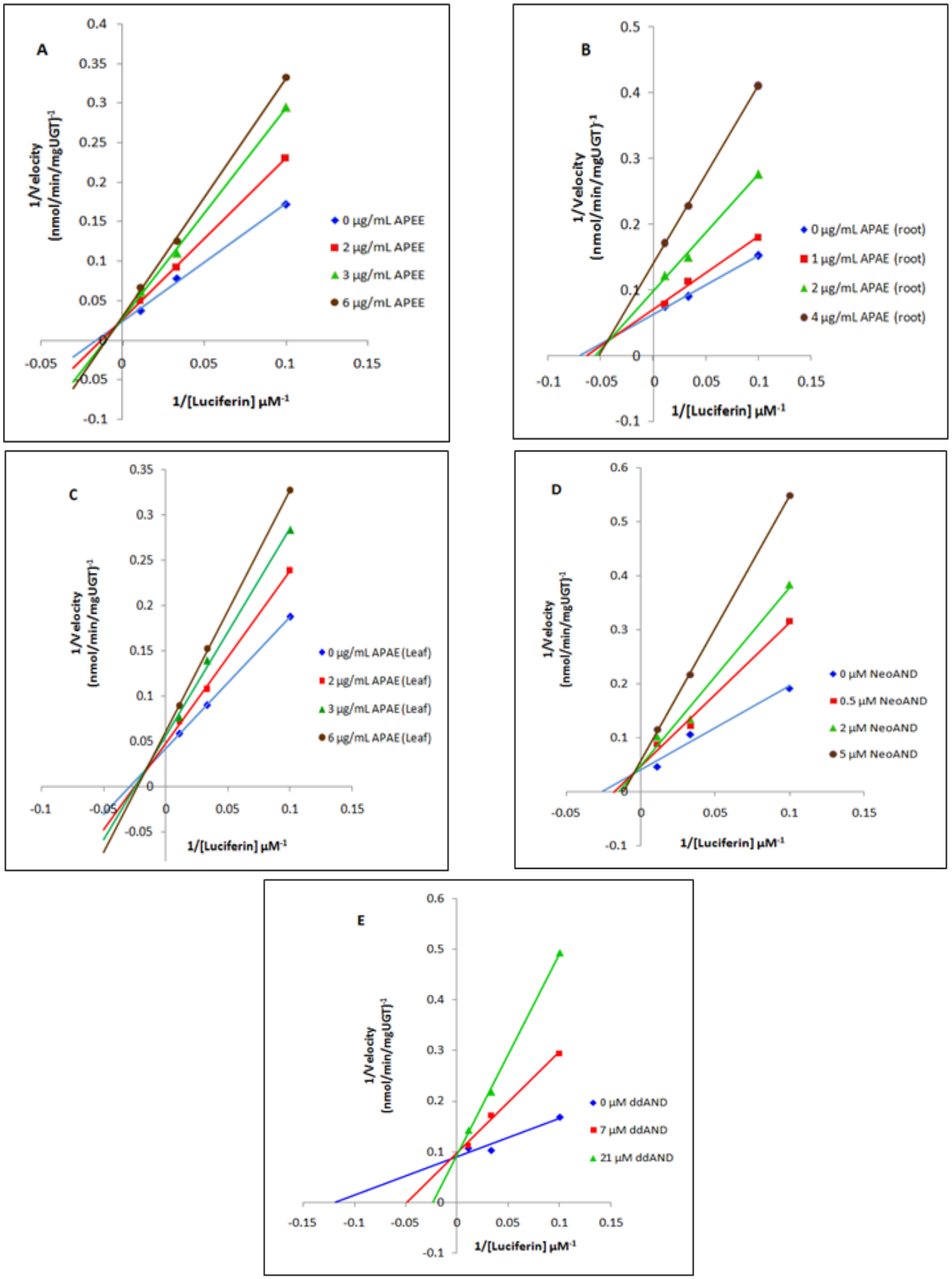

Fig. 2: Lineweaver-Burk plots of human UGT1A1 inhibition by (A) AP ethanol extract, (B) AP aqueous extract (root), (C) AP aqueous extract (leaf), (D) neoandrographolide, and (E) 14-Deoxy-11,12-didehydroandrographolide. Each data point represents the mean of triplicate determinations.

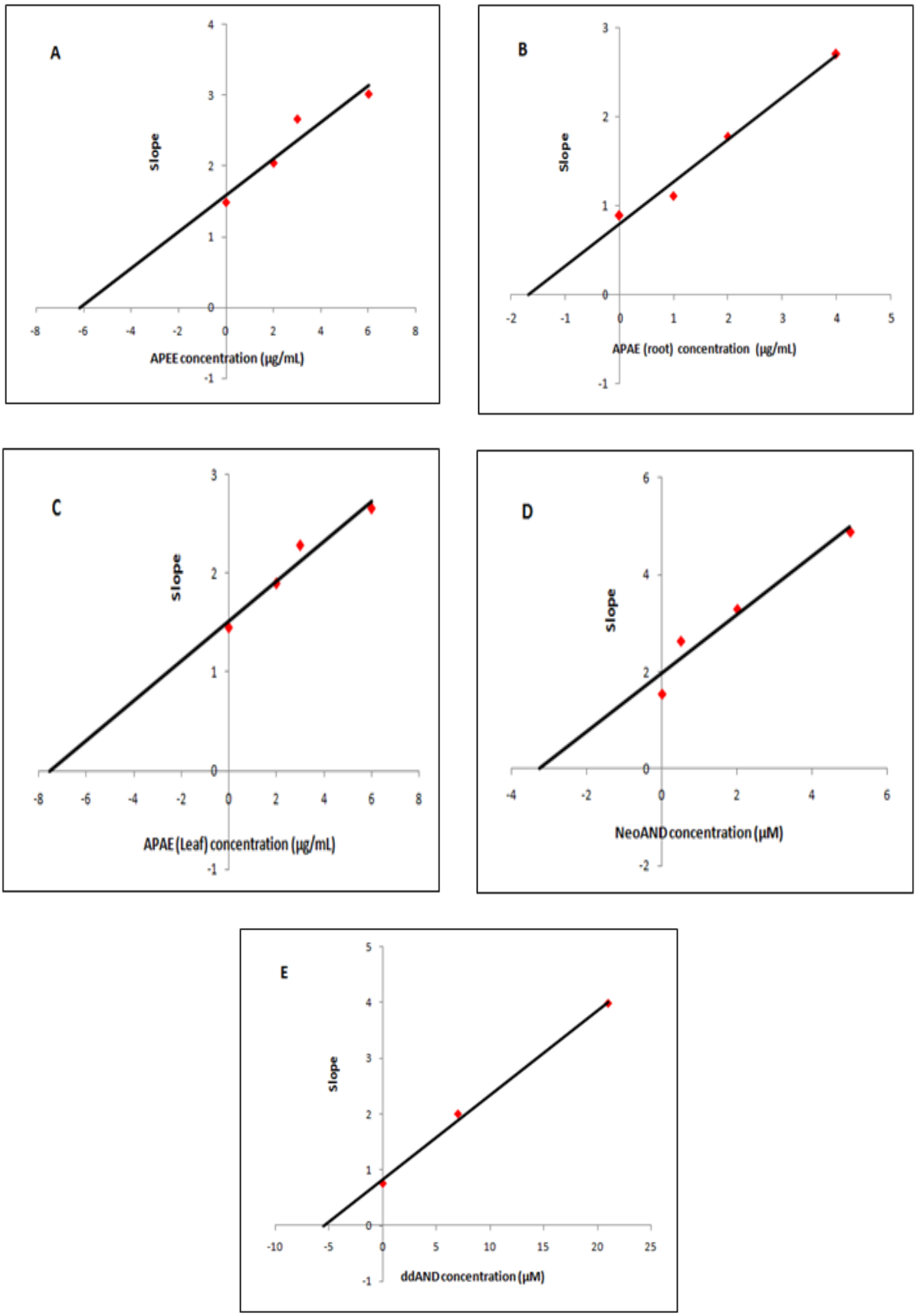

Fig. 3: Secondary plots of the slopes (from Lineweaver-Burk plots of UGT1A1 inhibition) *vs.* the concentrations of (A) AP ethanol extract, (B) AP aqueous extract (root), (C) AP aqueous extract (leaf), (D) neoandrographolide, and (E) 14-Deoxy-11,12-didehydroandrographolide. Each data point represents the mean of triplicate determinations.

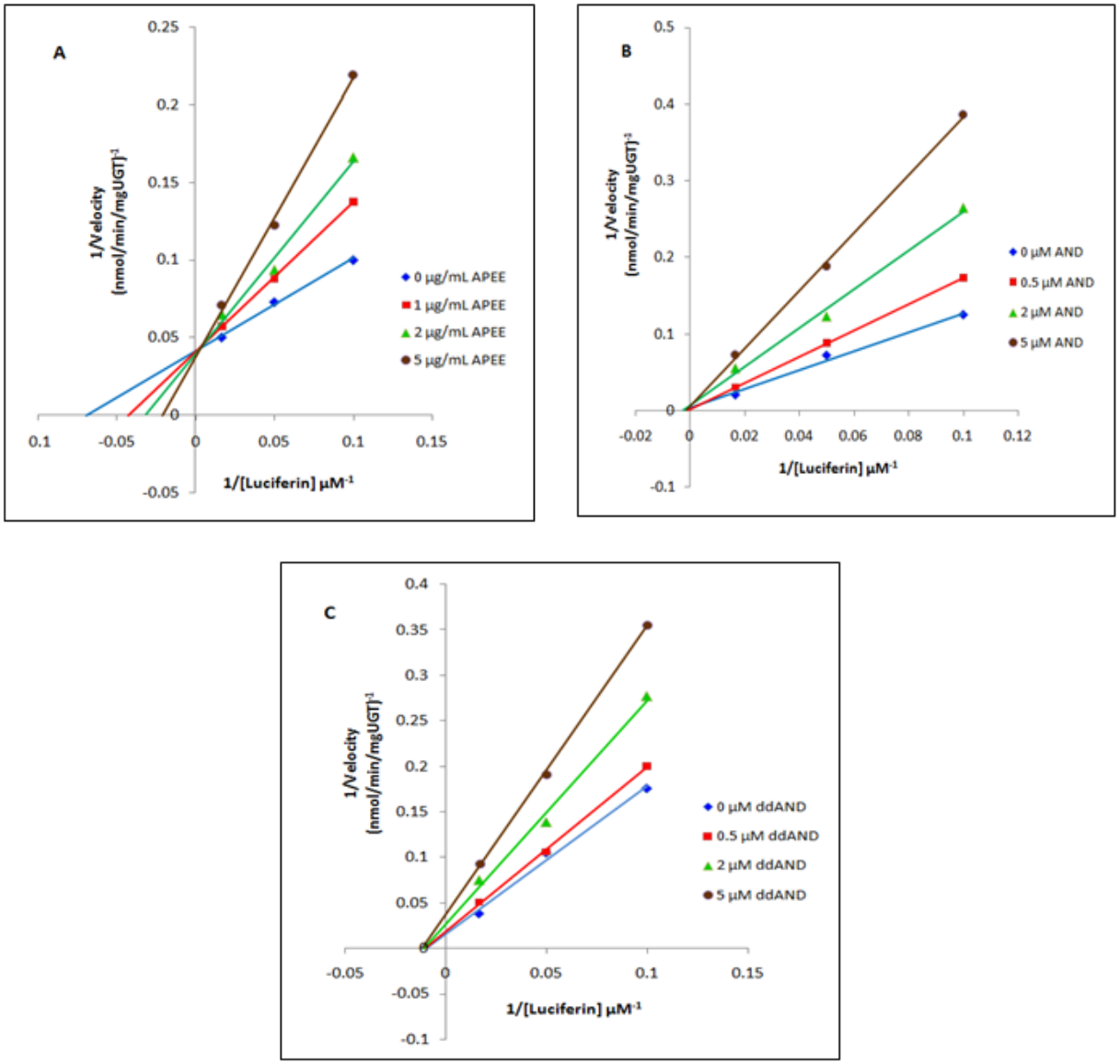

Fig. 4: Line weaver-Burk plots of human UGT2B7 inhibition by (A) AP ethanol extract, (B) andrographolide, and (C) 14-Deoxy-11,12-didehydroandrographolide. Each data point represents the mean of triplicate determinations.

Inhibitory effects of AP extracts and active constituents on UGT2B7

UGT2B7 activities were variedly inhibited by the extracts and constituents, exhibiting potent to mild degrees of inhibition, with IC_{50} 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 K*i* 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 (r^2) 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 IC_{50} 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.

A summary of the kinetic results presented in Table 2 illustrates differential effects in UGT isoforms inhibition among the AP extracts and constituents. AP ethanolic extract, AP aqueous (root) extract and AP aqueous (leaf) extracts potently inhibited UGT1A1, were best described by mixed inhibition models. On the other hand only AP ethanolic extract inhibited UGT2B7 with competitive mode. All constituents in general exhibited strong inhibition on UGT1A1 and UGT2B7 while none of them showed significant inhibitory effect on UGT1A4, consistent with the effects by the extracts. This variability of the inhibition of UGTs by the constituents may reflect a catalytic interaction, as UGT1A1 and UGT2B7 have been previously

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 exhibit 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 K*i* 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, epirubicin, 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

AP extract *	Content of active constituents (%) **		
	AND ***	NeoAND	ddAND
AP ethanol	15.60	4.90	nd
AP methanol	1.59	4.72	6.64
AP aqueous (root)	0.026	nd	nd
AP aqueous (stem)	0.40	0.13	0.40
AP aqueous (leaf)	0.046	0.64	nd

* AP: *Andrographis paniculata*, ** Mean of three determinations in which the results varied by 15% or less., ***AND: andrographolide, NeoAND: neoandrographolide, ddAND: 14-Deoxy-11,12-didehydroandrographolide, nd: not detectable under the assay condition.

To place into perspective the magnitude of UGT2B7 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 K*i* value of 2.9 µg/mL, suggesting that andrographolide might be mainly attributed to the UGT2B7 inhibition by AP ethanolic. On the basis of 14-Deoxy-11,12-didehydroandrographolide (ddAND) low K*i* 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 K*i* 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 propensity to interact with UGT2B7 substrate. The assessment of UGT1A1 inhibition by AP extracts demonstrated potent interaction in the order of decreasing effect; AP aqueous (root) > AP ethanolic > AP aqueous (leaf) as depicted by their respective K*i* values. Using similar estimate as that made for UGT2B7, all the three AP extracts display K*i* values in physiologically reachable concentration based on the estimated total plasma concentration of the extracts, which was higher than their K*i* 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 has 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 K*i* 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 neoandrographolide and ddAND content with their fairly moderate IC_{50} 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 diterpenes 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 *A. 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 significant 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, neoandrographolide 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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