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ORIGINAL ARTICLE

# *In vitro* Inhibitory Effects of *Andrographis paniculata, Gynura procumbens, Ficus deltoidea*, and *Curcuma xanthorrhiza* Extracts and Constituents on Human Liver Glucuronidation Activity

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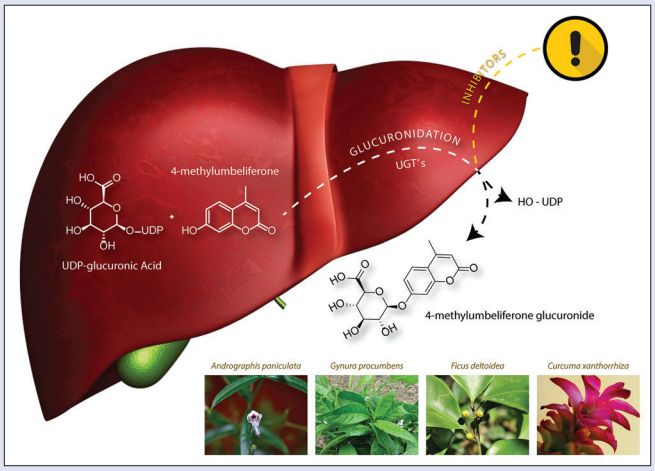
## ABSTRACT

**Background:** *Andrographis paniculata, Gynura procumbens, Ficus deltoidea* and *Curcuma xanthorrhiza* are commonly consumed as herbal medicines. However their effects on human liver glucuronidation activity are not yet evaluated. **Objective:** In this study, we evaluate the inhibitory Effects of Andrographis paniculata, Gynura procumbens, Ficus deltoidea and Curcuma xanthorrhiza extracts and their constituents on human liver glucuronidation activity. **Materials and Methods:** Herbal extracts (aqueous, methanolic and ethanolic extracts) and their constituents were incubated with human liver microsomes with the addition of UDPGA to initiate the reaction. Working concentrations of herbal extracts and their constituents ranged from 10 µg/mL to 1000 µg/mL and 10 µM to 300 µM respectively. IC50 was determined by monitoring the decrement of glucuronidation activity with the increment of herbal extracts or phytochemical constituent's concentrations. **Results:** All herbal extracts inhibited human liver glucuronidation activity in range of 34.69 µg/mL to 398.10 µg/mL whereas for the constituents, only xanthorrhizol and curcumin (constituents of *Curcuma xanthorrhiza*) inhibited human liver glucuronidation activity with IC50 of 538.50 and 32.26 µM respectively. **Conclusion:** In the present study, we have proved the capabilities of *Andrographis paniculata, Gynura procumbens*, Ficus deltoidea and *Curcuma xanthorrhiza* to interfere with *in vitro* glucuronidation process in human liver microsomes.

**Key words:** *Andrographis paniculata, Gynura procumbens, Ficus deltoidea, Curcuma xanthorrhiza,* UGT Inhibition

## SUMMARY

- This study documented the capabilities of *Andrographis paniculata, Gynura procumbens, Ficus deltoidea* and *Curcuma xanthorrhiza* to inhibit human liver glucuronidation activity which may affect the metabolism of therapeutic drugs or hazardous toxicants that follow the same glucuronidation pathway.




**Abbreviations used:** UGT: Uridine 5'-diphospho-glucuronosyltransferase; 4-MU: 4-methylumbelliferone; IC50: Half Maximal Inhibitory Concentration; Km: Michaelis constant; Vmax: Maximum velocity.

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## INTRODUCTION

Uridine 5'-diphospho-glucuronosyltransferase (UGT) is a superfamily of protein enzymes that are responsible for the metabolism of endobiotics and xenobiotics in many organisms including humans. UGT enzymes play a major role in catalyzing a conjugation process known as glucuronidation. During glucuronidation, a glucose-derived moiety, glucuronic acid is conjugated to a suitable functional site (hydroxyl, carboxyl, carbonyl, sulfhydryl, and amine but not hydroxyl from sugar ring) on a substrate modulated by UGT proteins. In general, this metabolic process generates a highly soluble inactive metabolite which renders excretion process in the body [Figure 1]. Today, UGT enzymes are classified under the group of drug-metabolizing enzyme with purpose to detoxify poisonous compounds from interfering with human biological process.

UGT enzymes display a broad and overlapping substrate specificities, which are common features for drug-metabolizing enzymes.[1] Human UGTs are divided into two families, UGT1 and UGT2. These two families originate from two separated genes located on chromosome 2q37 and 4q13, respectively, which encode a number of different proteins yet, still perform the same glucuronidation function.[2] Genetic mutations in UGT sequences or inhibition of UGT metabolic activities may cause serious threats, especially in metabolizing environmental toxicants or native endobiotics, for example bilirubin, a by-product of hemoglobin catabolism which can only be removed through conjugation with glucuronic acid.[3]



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As UGT proteins display a group of superfamily enzymes, a suitable probe substrate need to be selected in such a way that each UGT member can metabolize that particular probe. mRNA expression studies of UGT families in normal human tissues indicate that human liver cells were able to express UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, UGT2B4, UGT2B7, UGT2B10, UGT2B11, UGT2B15, and UGT2B17 subfamily proteins or commonly referred to as isoforms.[4] Uchaipichat *et al.*[5] reported a fluorescence molecule; 4-methylumbelliferone (4-MU) was able to be glucuronidated by most of UGT isoforms expressed in the human liver with the exception of UGT2B10 and UGT2B11. For that reason, 4-MU has been recognized to be the best probe substrate for monitoring overall hepatic UGTs activity in humans.[6]

Hitherto, herbal medicines stand almost exclusively in a class of its own, especially in today's world of modern medicines. Herbs traveled through a long and rich history, particularly in traditional medication and food making. Often cited as "natural," herbal medicines are considered to be safer than human-made synthetic medicines. However, under the Bill of Dietary Supplement Health and Education Act 1994 (DSHEA), herbs belong to a class of dietary supplement (rather than pharmaceutical drugs) which severely limits the US Food and Drug Administration's (FDA) ability to regulate this class of substances. Unlike controlled drugs, medicinal herbs under the Bill of DSHEA require no proof of efficacy, no proof of safety, and set no standards for quality control for any product to be labeled as a dietary supplement. Since then, productions of herbal supplements by the industries become viral. Many herbal products flooded the market which in turn will increase the possibility of herbal products intake as an alternative to conventional modern medicines or even worst, taking both of them at the same time and causing a greater danger.[7]

*Andrographis paniculata* from Acanthaceae family is indigenous to India, China, and South East Asia.[8] Traditionally, it is used for treating common cold, influenza, gastric disorder, liver diseases, and AIDS.[9-14] *Gynura procumbens* is a fast-growing evergreen shrub and it belongs to Compositae family. It is found in various parts of Asia, and it is frequently used for treating eruptive fever, migraine, rash, constipation, hypertension, diabetes mellitus, and cancer.[15,16] *Ficus deltoidea* from Moraceae family is an epiphytic shrub, and it is widely distributed in South East Asia. This plant is used to treat pneumonia, gout, high blood pressure, diarrhea, and skin infections. It is also used as an aphrodisiac to enhance fertility in males.[17] *Curcuma xanthorrhiza* is one of the members of Zingiberaceae family, and it originates from Indonesia. It is widely grown in Thailand, Sri Lanka, Philippines, and Malaysia. The rhizomes of this plant are well known to be effective against gallstone, jaundice, stomach illness, and possess anticancer properties.[18]

Concomitant consumption of herbal supplements along with pharmaceutical drugs is an inclining trend in the last decade.[19,20] It is found herbal phytochemicals are able to undergo glucuronidation process and this give rise to possibility of herb–drug interaction with other pharmaceutical drugs assuming both entities are involved in the same glucuronidation pathway.[21] In this study, we attempt to find the inhibitory effects of four selected medicinal herb extracts; *A. paniculata*, *G. procumbens*, *F. deltoidea*, and *C. xanthorrhiza* on human liver glucuronidation activity. There will be three different extracts (aqueous, methanolic, and ethanolic) for each selected test-herb and nine phytochemical constituents originated from these four herbs are chosen for inhibition assay [Table 1]. Inhibitor concentration at a point where it inhibits glucuronidation activity of 4-MU by half represent the half maximal inhibitory concentration ($IC_{50}$) value and this signifies the inhibitory potency of selected plant extracts and their phytochemical constituents on human liver glucuronidation activity.

## MATERIALS AND METHODS

4-MU sodium salt (*M1508-SIGMA*), 4-methylumbell iferone-β-D-glucuronide (4-MUG) (*M9130-SIGMA*), uridine 5'-diphosphoglucuronic acid trisodium salt (UDPGA) (*U6751-SIGMA*), andrographolide (*365645-SIGMA*), neoandrographolide (*49879-SIGMA*), astragalin (*79851-SIGMA*), kaempferol-3-O-rutinoside (*90242-SIGMA*), vitexin (*49513-SIGMA*), isovitexin (*17804-SIGMA*), curcumin (*C1386-SIGMA from turmeric powder*), TRIZMA® preset crystal pH 7.1 at 37°C (TRIS), Triton X-100, and magnesium chloride ($MgCl_2$) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Triethylamine was purchased from R and M Marketing (Essex, UK). Trichloroacetic acid (TCA) was purchased from Acros Organics (New Jersey, USA). Perchloric acid 70% was purchased from Merck (Darmstadt, Germany). HPLC grade acetonitrile was purchased from RCI Labscan Limited (Bangkok, Thailand). Pure xanthorrhizol and curcumene were isolated from *C. xanthorrhiza* hexane extract based on our previous work.[22] Human liver microsomes were purchased from Sigma-Aldrich, St. Louis, MO, USA (Product No. M0567). This product contains a mixture of liver microsomes pooled from different individual human donors. The donors were human males of mixed age. The donors were in various states of health; however. each liver was tested negative for HIV 1 and 2, HTLV 1 and 2, and hepatitis B and C (Sigma M0567 Product Information).

### Method

#### *Preparation of herbal extracts*

*G. procumbens* (leaves), *F. deltoidea* (leaves), *and C. xanthorrhiza* (rhizomes) were prepared under similar procedure in

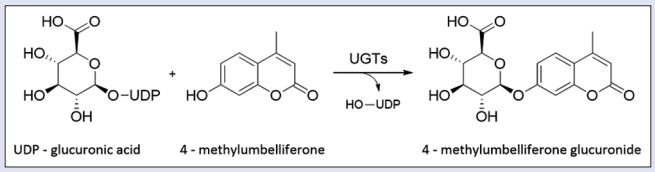


**Figure 1:** Glucuronidation reaction between uridine 5'-diphospho-glucuronic acid with 4-methylumbelliferone catalyzed by the UGT enzymes to produce 4-methylumbelliferone glucuronide as a metabolite. UGT: Uridine 5'-diphospho-glucuronosyltransferase

**Table 1:** Phytochemical composition of herbal constituents in each selected medicinal herbs

| Herbal extracts | Weight percentage, w/w (%) | | | References |
|---|---|---|---|---|
| | AE | ME | EE | |
| *Andrographis paniculata* | | | | |
| Andrographolide | 0.05 | 1.59 | 15.60 | Abidin *et al.*[34] |
| Neoandrographolide | 0.64 | 4.72 | 04.90 | |
| *Gynura procumbens* | | | | |
| Astragalin | 0.00 | 7.41 | 05.20 | Afandi *et al.*[27] |
| Kaempferol-3-O-rutinoside | 0.00 | 1.06 | 00.45 | |
| *Ficus deltoidea* | | | | |
| Vitexin | 0.41 | 2.12 | 00.40 | Zulkiffli[28] |
| Isovitexin | 0.62 | 1.84 | 00.62 | |
| *Curcuma xanthorrhiza* | | | | |
| Xanthorrhizol | 3.41 | 14.59 | 22.89 | Halim *et al.*[22] |
| Curcumene | 2.11 | 2.81 | 13.60 | |
| Curcumin | 2.05 | 6.96 | 10.55 | Unpublished data |

AE: Aqueous extract; ME: Methanolic extract; EE: Ethanolic extract

our laboratory. For aqueous extract, each plant (100 g powdered) was soaked in 1 L of stirred water bath at 60°C for 3 h, and the solution was filtered before freeze-drying step. For methanolic extract (ME) and ethanolic extract (EE), each plant (100 g powdered) was macerated for 3 days under dark condition in 3 L of respective solvents. Macerated solutions were filtered and dried using rotary evaporator. For *A. paniculata* (leaves), all the three different extracts were generously provided by Prof. Chan Kit Lam from the School of Pharmaceutical Sciences, Universiti Sains Malaysia. Dried extracts were kept in a dry and dark place until further use.

### *HPLC separation method*

HPLC method was adopted from previous work by Haron and Ismail[23] but with some modifications. Analysis was performed using Jasco LC-2000Plus Series HPLC System. This instrument consists of a hardware interface (LC-NetII/ADC), a quaternary gradient pump (PU-2089PLUS), an autosampler (AS-2055PLUS), and a column thermostat (CO-2065PLUS) which is hyphenated to a ultraviolet/visible spectrophotometric detector (UV-2070 Plus). Analyte separations were done using a reversed-phase column (Phenomenex Gemini NX C18 4.6 mm × 150 mm 5.0 µ) thermostated around 40°C under gradient elution mode at 1.0 mL/min for a total run time of 10 min. Separation gradient profiles are provided as follows; two mobile phase reservoirs were used which consist of reservoir A (HPLC Grade Acetonitrile) and reservoir B (10 mM triethylamine adjusted to pH 2.5 using perchloric acid). At the first 3 min, mobile phase compositions of A to B were kept constant at a ratio of 14:86. Then, composition of A was ramped up to a ratio of 90:10 from 3 to 4 min and finally reverted to its beginning ratio of 14:86 at 4 min to be prepared for the next sample injection. Total analysis time per sample was 8 min with 6 min run time and 2 min post-time. HPLC eluents were monitored under ultraviolet region at 316 nm wavelength, and the retention time of 4-MUG was 4.0 min and 4-MU eluted at post-time region along with other compounds that may still retain at the end of 6 min run time.

### *Optimization of incubation assay*

Incubation assay was optimized by observing the linearity of metabolite formation with respect to time of incubation, protein content, and detergent concentration (Triton X-100). The final optimized method was incubated for 15 min at 37°C in a total volume of 200 µL which consist of 0.1 mg/mL of human protein microsomes, 0.2 µL/mg protein of Triton X-100, 0.1 mM of 4-MU, 10 mM of $MgCl_2$, 50 mM of TRIS (pH 7.1 at 37°C), and 5 mM of UDPGA. Substrate concentration was chosen according to the values of kinetic constants, Michaelis constant ($K_m$), and maximum velocity ($V_{max}$) obtained from the experiment based on classical Michaelis–Menten kinetics. Rate of enzymatic reaction was measured under initial velocity where less than 10% of the substrate has been depleted. It is assumed that under these conditions, the substrate concentration does not significantly change and the reversed reaction does not contribute to the reaction rate.

### *Determination of half maximal inhibitory concentration*

$IC_{50}$ was determined by monitoring the decrement of enzymatic activity with the increment of herbal extracts or phytochemical constituent's concentrations. Working concentrations of herbal extracts and phytochemical constituents ranged from 10 µg/mL to 1000 µg/mL and 10 µM to 300 µM, respectively. A premix enzyme source containing protein microsomes, Triton X-100, 4-MU, $MgCl_2$, TRIS, and working concentration range of herbal extracts or constituents were preincubated on ice for 15 min. Then, the mixture was preheated in 37°C water bath for another 15 min before UDPGA was introduced to the mixture to initiate the reaction. Incubations were carried out for 15 min under shaking water bath, and reaction was terminated by the addition of 100 µL ice-cold TCA (20%). Incubations were allowed to stand on ice for 30 min to fully terminate the catalytic activity of the enzymes. Samples were centrifuged at 10,000 RCF for 20 min before injected to HPLC (5 µL injection volume) for further analysis.

### *Data analysis*

Results are represented as mean ± standard error of mean for three independent measurements ($n$ = 3). $IC_{50}$ values were calculated using GraphPad Prism 6.01 by GraphPad Software, Inc. under dose–response inhibition (log [inhibitor] vs. normalized response – variable slope) model. Data sets were normalized with respect to control incubation (100% activity) for $IC_{50}$ measurements. $K_m$ and $V_{max}$ were determined using the same software under enzyme-kinetic inhibition (substrate inhibition) model.

## RESULTS

### Enzyme kinetics

Determination of kinetic constant $K_m$ and $V_{max}$ was done by monitoring the enzymatic velocity at different levels of substrate concentration. Classical Michaelis–Menten kinetics states, at a fixed amount of enzyme, reaction velocity increases when more substrates are added into the enzymatic system until a maximum velocity is achieved. At this state, all of the enzyme's active sites are occupied by the presence of saturated substrate. In contrast to classical Michaelis–Menten model, glucuronidation of 4-MU was found to follow substrate inhibition model where reduction in $V_{max}$ value occurs when a large amount of substrate was introduced to the enzymes. The values of $K_m$ and $V_{max}$ obtained in this study are 0.125 ± 0.03 mM and 71.20 ± 5.39 nmol/mg/min, respectively [Figure 2]. These values are in agreement with results obtained by Hanioka *et al.*[24] With exception that, our findings suggest atypical kinetic behavior on 4-MU glucuronidation in human liver. We chose 0.1 mM of 4-MU as the substrate concentration for our control parameter which generates a velocity of 30.44 ± 1.84 nmol/mg/min.

### Effects of *Andrographis paniculata* on human liver glucuronidation activity

Inhibition of human liver glucuronidation activity was done by performing glucuronidation reaction of 4-MU catalyzed by UGT enzymes from liver microsomes in the presence of herbal extracts or constituents. Three solvents with different polarities (water, methanol, and ethanol) were chosen as these solvents are extensively used in herbal medicine preparation. Different types of solvents cause phytochemical content for each extract to be diversified hence provide different degrees of inhibition. Aqueous, ME, and EE of *A. paniculata* inhibited human liver glucuronidation activity with $IC_{50}$ values of 398.10 µg/mL, 182.90 µg/mL, and 60.44 µg/mL, respectively. On the other hand, *A.* paniculata's main constituents, andrographolide and neoandrographolide, showed no significant inhibition on human liver glucuronidation activity [Figures 3 and 4].

### Effects of *Gynura procumbens* on human liver glucuronidation activity

Inhibition of *G. procumbens* extracts followed similar trends as *A. paniculata*. The lowest rank of inhibition started from aqueous extract then MEs followed by the EE. The $IC_{50}$ values of aqueous, ME, and EEs were 347.40 µg/mL, 137.70 µg/mL, and 64.26 µg/mL, respectively. Phytochemical constituents of *G. procumbens*, astragalin, and kaempferol-3-O-rutinoside showed no significant inhibition on human liver glucuronidation activity [Figures 3 and 4].

## Effects of *Ficus deltoidea* on human liver glucuronidation activity

While *A. paniculata* and *G. procumbens* displayed similar trend of inhibition, *F. deltoidea,* however, showed different inhibition ranks where ME inhibited human liver glucuronidation activity the most ($IC_{50}$ = 48.65 µg/mL) compared to aqueous and EEs. Aqueous extract follows the same trend like other plant extracts where it weakly inhibited glucuronidation activity in human liver ($IC_{50}$ = 164.60 µg/mL). Second inhibition rank was shown by EE which inhibited 50% of human liver glucuronidation activity at $IC_{50}$ value of 67.79 µg/mL. *F. deltoidea*'s phytochemical constituents, vitexin and isovitexin do not affect the glucuronidation activity in human liver [Figures 3 and 4].

## Effects of *Curcuma xanthorrhiza* on human liver glucuronidation activity

*C. xanthorrhiza* EE was found to be the most potent inhibitor among all studied plant extracts. It inhibited human liver glucuronidation activity at $IC_{50}$ value of 34.69 µg/mL. ME potently inhibited human liver glucuronidation activity ($IC_{50}$ = 37.14 µg/mL) but slightly higher compared to EE. Aqueous extract inhibited human liver glucuronidation activity with an $IC_{50}$ value of 271.10 µg/mL. While the constituents from other plant extracts did not show any significant inhibition, two out of three constituents from *C. xanthorrhiza* inhibited glucuronidation activity which are xanthorrhizol ($IC_{50}$ = 583.50 µM) and curcumin ($IC_{50}$ = 32.26 µM). Curcumene did not affect the glucuronidation activity in human liver. Summary of $IC_{50}$ values of all plant extracts and its phytochemical constituents is shown in Table 2.

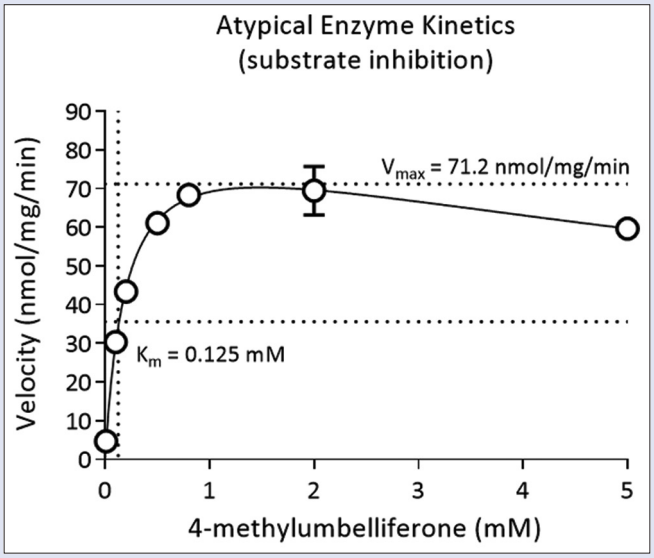


**Figure 2:** Enzyme kinetic analysis of 4-MU glucuronidation in human liver microsomes catalyzed by UGT enzymes. Glucuronidation of 4-MU follows atypical enzyme kinetic model where the substrate itself act as an inhibitor after maximum velocity is achieved. Unlike the typical model of Michaelis–Menten kinetic, maximum velocity value in this model will never reach a plateau state when a high amount of substrates are introduced into the system. 4-MU: 4-methylumbelliferone; UGT: Uridine 5′-diphospho-glucuronosyltransferase

**Table 2:** Inhibitory effects of herbal extracts and their phytochemical constituents on human liver glucuronidation activity

| Herbal extracts and its constituents | $IC_{50}$ (µg/mL) | $IC_{50}$ (µM) |
|---|---|---|
| *Andrographis paniculata* | | |
| Aqueous extract | 398.10±1.20 | - |
| ME | 182.90±1.12 | - |
| EE | 60.44±1.09 | - |
| Andrographolide | NA* | NA* |
| Neoandrographolide | NA* | NA* |
| *Gynura procumbens* | | |
| Aqueous extract | 347.40±1.21 | - |
| ME | 137.70±1.06 | - |
| EE | 64.26±1.08 | - |
| Astragalin | NA* | NA* |
| Kaempferol-3-O-rutinoside | NA* | NA* |
| *Ficus deltoidea* | | |
| Aqueous extract | 164.60±1.09 | - |
| ME | 48.65±1.09 | - |
| EE | 67.79±1.06 | - |
| Vitexin | NA* | NA* |
| Isovitexin | NA* | NA* |
| *Curcuma xanthorrhiza* | | |
| Aqueous extract | 271.10±1.07 | - |
| ME | 37.14±1.04 | - |
| EE | 34.69±1.12 | - |
| Xanthorrhizol | 117.55±0.25 | 538.50±1.14 |
| Curcumene | NA* | NA* |
| Curcumin | 11.88±0.39 | 32.26±1.06 |

*Not available: $IC_{50}$ value is very high and not significant. Each result represents mean $IC_{50}$±SEM for triplicate measurements ($n$=3). Plant extracts are not expressed in molarity unit (µM), as more than one compound is present in each extract. $IC_{50}$: Half maximal inhibitory concentration; SEM: Standard error mean; ME: Methanolic extract; EE: Ethanolic extract

# DISCUSSION

It has come to our attention that a lot of herbal phytochemicals are able to undergo glucuronidation process and disrupts the metabolism of other pharmaceutical drugs or environmental toxicants which may follow the same glucuronidation pathway. Changes in drug efficacy or toxicity may occur as a result from herb–drug interaction. In the present study, four selected herbal extracts with therapeutic properties were screened for their effects on human liver glucuronidation activity.

*A. paniculata* inhibited human liver glucuronidation activity at different potencies depending on the type of solvents used for each extract. Water-soluble phytochemicals supposedly require no modification from metabolizing enzymes as they are able to dissolve in the urine to be excreted out from the body. This may be the major reason for weak inhibition caused by aqueous extract of this plant. In contrast, ethanol and methanol will extract more lipophilic phytochemicals as compared to water. In comparison between ethanol and methanol to extract lipophilic phytochemicals, ethanol is more effective since ethanol's hydrophobicity is greater than methanol. Lipophilic substances are more attracted toward metabolizing enzymes such as CYPs or UGTs.[25] As a result, EE of this plant potently inhibited human liver glucuronidation activity followed by methanolic than aqueous extracts. While the present study focused on general glucuronidation of human liver, our previous study which targets several UGT isoforms for inhibition studies with EE of *A. paniculata* had been done.[8] *A. paniculata* EE inhibited all of our studied human isoforms (UGT1A1, 1A3, 1A6, 1A7, 1A8, 1A10, 2B7, and 2B15) on 4-MU glucuronidation at $IC_{50}$ range of 1.70–30.0 µg/mL. It is important to point out the preparation of EEs in our previous study is through Soxhlet extraction technique which exhaustively extracts all phytochemicals that can dissolve in their respective solvent rather than simple maceration that restricted to solubility limit of most phytochemicals in a solvent. Lipophilic content in Soxhlet technique is higher; therefore, it shows a greater inhibition on glucuronidation activity compared to maceration technique. Nevertheless, maceration technique was chosen in this study as it resembles well to classical preparation of traditional herbs worldwide.

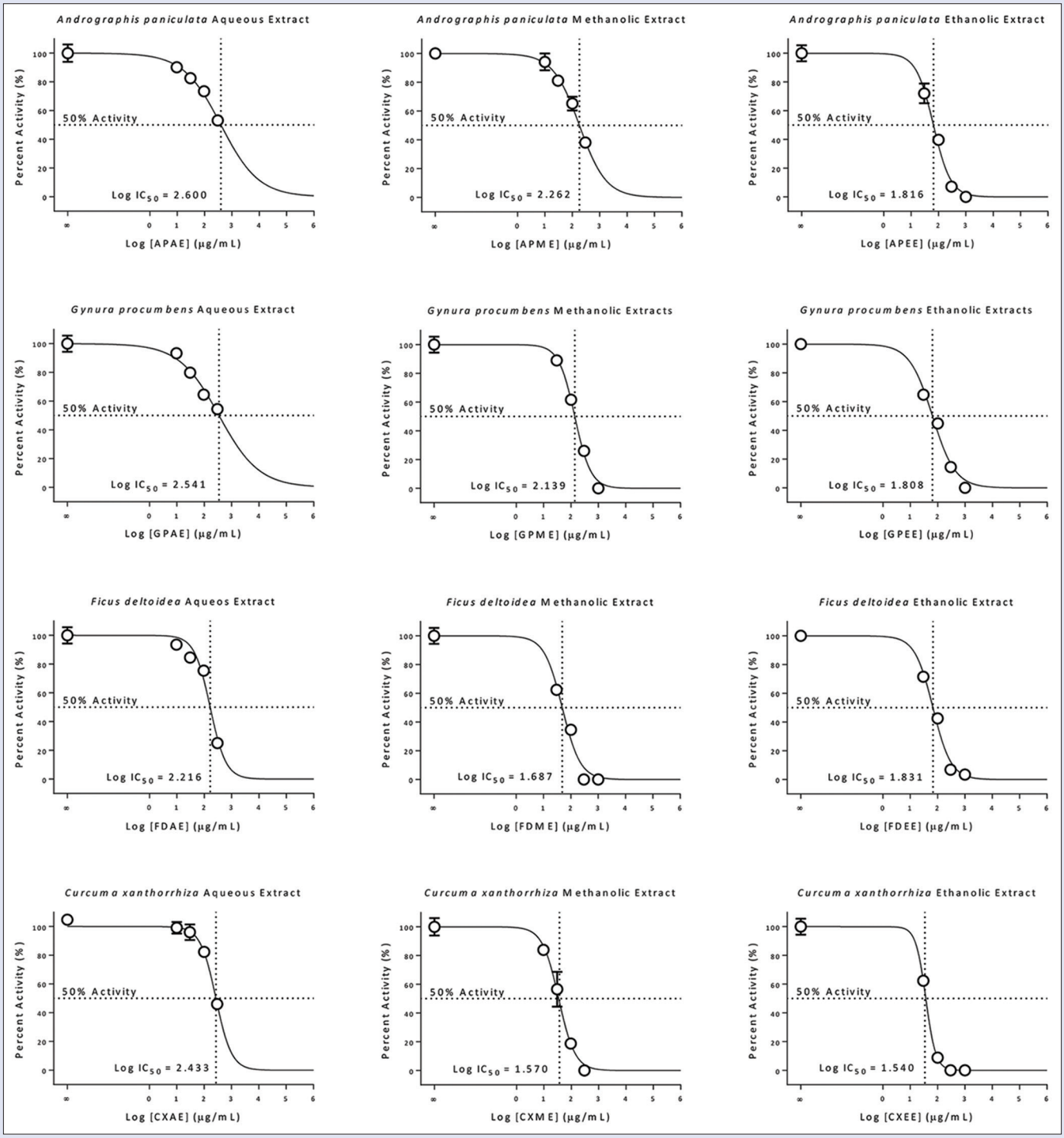


**Figure 3:** Half maximal inhibitory concentration profiles of four different plant extracts – *Andrographis paniculata*, *Gynura procumbens*, *Ficus deltoidea*, and *Curcuma xanthorrhiza* with different solvent extractions on human liver glucuronidation activity. ∞ symbolizes zero concentration of inhibitor (Log[0] = ∞) which represent 100% activity of control parameter

Our previous study[8] revealed *A. paniculata*'s main constituent, andrographolide, selectively inhibited human UGT2B7 isoform while not affecting other isoforms (UGT1A1, 1A3, 1A6, 1A7, 1A8, 1A10, and 2B15). However, in the present study, we found that andrographolide did not affect the activity of human liver glucuronidation activity. One plausible explanation is that selective inhibition of UGT2B7 activity by andrographolide in human liver is masked by the superior activity of 4-MU metabolic conversion by UGT1A6. This viewpoint is based on evidence from Uchaipichat *et al.*[5] that reported metabolism of 4-MU is predominantly monopolized by UGT1A6 ($V_{max}$ = 143 897 pmol/mg/min) compared to UGT2B7 ($V_{max}$ = 168 pmol/mg/min) which clearly displayed thousand-fold differences in activity. Neoandrographolide, on the other hand, did not inhibit human liver glucuronidation activity, and this may be due to the lack of glucuronidation site on its molecular structure [Figure 5].

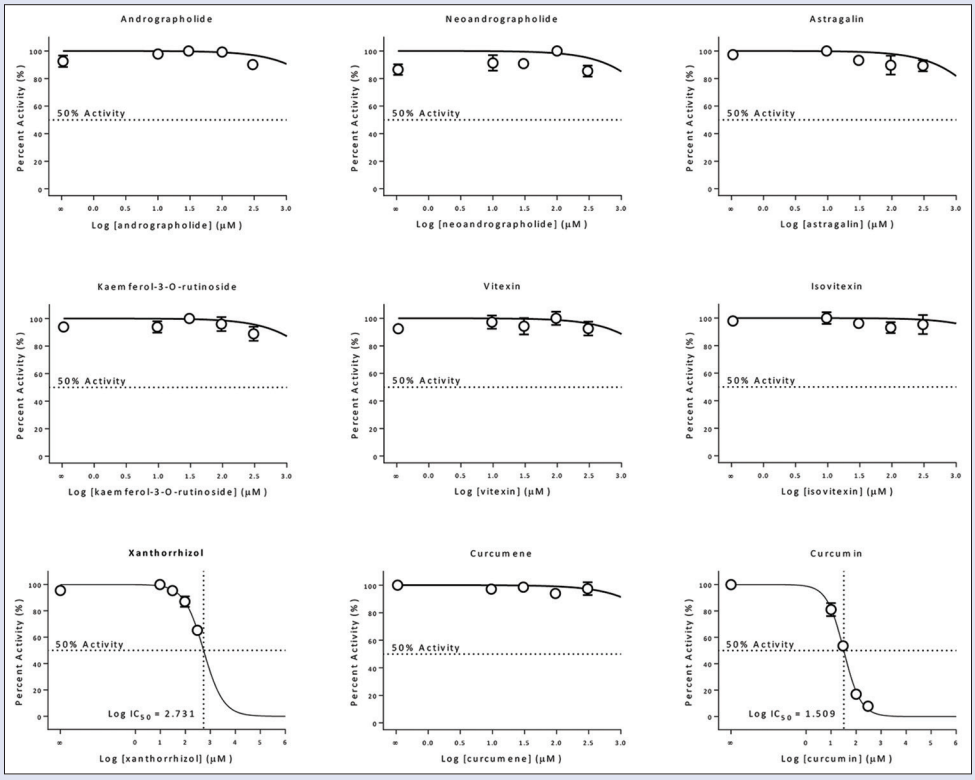


**Figure 4:** Half maximal inhibitory concentration profiles of plant extract phytoconstituents of *Andrographis paniculata*, *Gynura procumbens*, *Ficus deltoidea*, and *Curcuma xanthorrhiza* on human liver glucuronidation activity. ∞ symbolizes zero concentration of inhibitor (Log[0] = ∞) which represent 100% activity of control parameter

For *G. procumbens*, inhibition of each extract (aqueous, methanolic, and ethanolic) follows the same pattern as *A. paniculata* where the most potent inhibitor was EE, followed by ME and then aqueous extract. We concluded that EE contains the highest content of lipophilic phytochemicals that may have caused potent inhibition on human liver glucuronidation activity. It is also thought the strong inhibitory effect of plant extracts on glucuronidation activity may be due to its high flavonoid content. Many flavonoids, for example, quercetin and kaempferol are substrates for UGT enzymes,[26] and they may compete with other UGTs substrate on the enzyme's active site to cause inhibition. We determined the total flavonoid content of *G. procumbens* for all three extracts. The total flavonoid content of this plant in increasing order is – aqueous extract < methanolic extract < ethanolic extract.[27] These results are in parallel with our inhibition ranks for *G. procumbens* extracts in the present study.

Astragalin and kaempferol-3-O-rutinoside [Figure 6] are both derivatives of kaempferol. Although kaempferol is a substrate for UGT enzymes, the presence of its derivatives did not inhibit the human liver glucuronidation activity. As a matter of fact, these two derivatives are metabolic products of kaempferol biosynthesis pathway. Plants tend to transfer glucose moiety for their conjugation metabolism rather than to transfer glucuronic acid as in mammals.[2] Thus, as metabolites, astragalin and kaempferol-3-O-rutinoside, are not supposedly required to be re-metabolized through conjugation with glucuronic acid, and this is proved by our findings. To date, no studies have been done on the effects of this plant toward UGT isoforms which then limits our discussions up to this level.

*F. deltoidea* also inhibited human liver glucuronidation activity. Unlike other plants that showed highest inhibition for their EEs, highest inhibition for *F. deltoidea* was shown by its ME. This anomaly can be explained by quantifying the total flavonoid content of this plant. It was revealed that ME contains the highest level of flavonoids among other extracts of *F. deltoidea*. The total flavonoid content of this plant can be listed in increasing order as follows – aqueous extract < ethanolic extract < methanolic extract.[28] Although aqueous extract of this plant is the weakest inhibitor compared to ME and EE, its inhibition potency among other plants aqueous extracts is relatively the highest ($IC_{50}$ = 164.60 μg/mL) and acceptably comparable to both MEs from *A. paniculata* ($IC_{50}$ = 182.90 μg/mL) and *G. procumbens* ($IC_{50}$ = 137.70 μg/mL). Due to this, home-made preparation of *F. deltoidea* herbal extract (boiled with water) may inflict a higher degree of inhibition on human glucuronidation activity.

Further inhibition studies of phytochemical constituents of this plant on UGT enzymes indicated neither vitexin nor isovitexin inhibit human liver glucuronidation activity. Vitexin and isovitexin [Figure 7] are metabolites generated from metabolism of apigenin in plant biosynthesis pathway. Apigenin, found in many plants, is a phytochemical compound belonging to flavonoid class which is extensively metabolized by UGT enzymes.[29] Even though vitexin and isovitexin are derivatives from apigenin, both these compounds do not affect the activity of human liver glucuronidation; thus, we suggest that both these derivatives do not take part in glucuronidation process. Similarly, to astragalin from *G. procumbens*, vitexin and isovitexin are glucoside forms of apigenin, and they do not require structure modification by UGTs to be eliminated out from the body.

We have also determined the inhibitory effect of *C. xanthorrhiza* on human liver glucuronidation activity. EE was the most potent inhibitor on glucuronidation activity followed by methanolic then aqueous

extract which is consistent with our assumption that higher content of lipophilic phytochemicals in EE contributes to the inhibition of glucuronidation activity. In fact, EE of *C. xanthorrhiza* is the most potent inhibitor among all plant extracts in this study which closely followed by its ME. *C. xanthorrhiza* aqueous extract weakly inhibited human liver glucuronidation activity. Low flavonoid content[22] might be another reason for weak inhibition caused by this aqueous extract.

While no significant inhibitions observed by phytochemical constituents from other plants in this study, a few constituents from *C. xanthorrhiza* displayed some significant inhibitions. We have examined the inhibitory effects of three phytochemical constituents (xanthorrhizol, curcumene, and curcumin) from this plant. Recently, Salleh[30] reported that xanthorrhizol inhibited both UGT1A1 and UGT2B7 isoforms activity while curcumene inhibition was negligible. On the other hand, our study shows that xanthorrhizol moderately inhibited human liver glucuronidation activity whereas curcumene showed no inhibition. These preliminary results indicate that xanthorrhizol may possess the ability to be glucuronidated directly by UGTs forming glucuronide conjugates and further studies are necessary to conform this. We assume that curcumene do not participate in conjugation process by UGTs as there is no glucuronidation site on its hydrocarbon skeletal structure [Figure 8]. Unlike xanthorrhizol and curcumene which are still lacking in glucuronidation studies, curcumin, however, is more established to an extent where it has reached the stage of human clinical trial phase.[31]

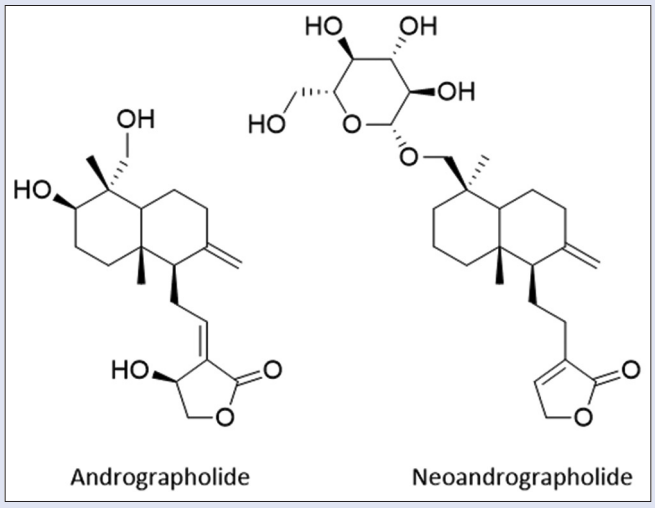


**Figure 5:** Phytochemical constituents, andrographolide and neo-andrographolide from *Andrographis paniculata* plant species

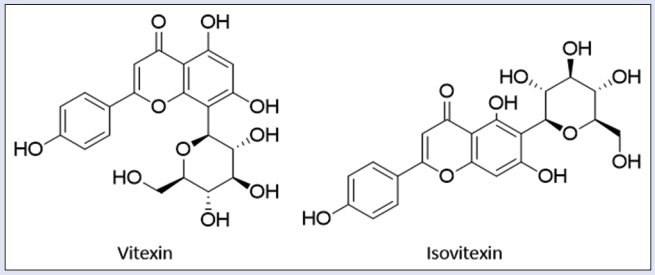


**Figure 7:** Phytochemical constituents, vitexin and isovitexin from *Ficus deltoidea* plant species

Curcumin is the principal compound responsible for the yellow color of *C. xanthorrhiza*'s rhizome part. Previous studies had demonstrated curcumin is poorly absorbed by the body.[32] Low bioavailability of curcumin is primarily caused by rapid metabolism of this compound through glucuronidation process in the liver and intestine.[33] In this study, inhibition by curcumin is potent as relatively small amount of curcumin is required to inhibit glucuronidation activity in human liver. Based on this finding, we believe that curcumin is the main constituent responsible for the inhibition on human liver glucuronidation activity by *C. xanthorrhiza* plant extracts.

## CONCLUSION

Herbal medicines are truly remarkable as they stand to be one of the most preferred medication treatments till date. Contrary to popular belief, consumptions of herbal medicines are not entirely safe or harmless. They are perceived to be risk-free as they are originated from natural product sources. Although these products do possess some potential benefits, they are capable of interacting with other drugs to cause toxicity. In the present study, we have proved the capabilities of several herbal medicines to interfere with *in vitro* glucuronidation process in human liver microsomes. To presume whether the herbs will bring an

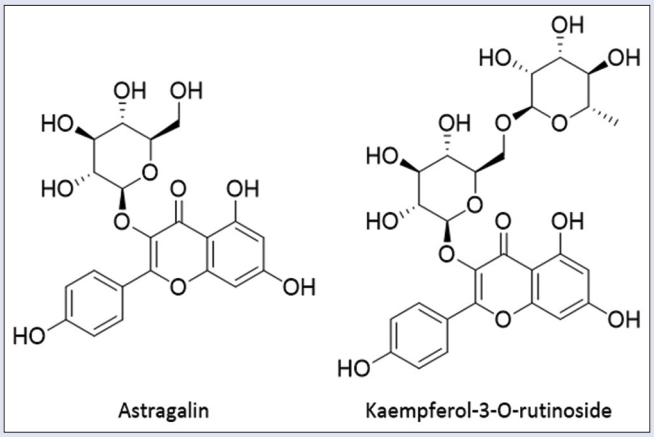


**Figure 6:** Phytochemical constituents, astragalin and kaempferol-3-O-rutinoside from *Gynura procumbens* plant species

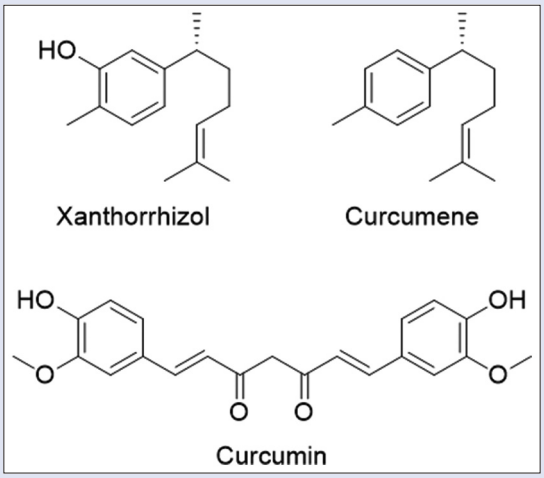


**Figure 8:** Phytochemical constituents, xanthorrhizol, curcumene, and curcumin from *Curcuma xanthorrhiza* plant species

actual inhibition in a real system is rather premature as *in vitro,* $IC_{50}$ data alone will not be sufficient. *In vivo* results will be a better estimate, whereas *in vitro* data will strengthen the findings. Nevertheless, the interaction risk that may cause by these herbs should not be ignored. Regardless whether it involves home-made preparation (aqueous extracts) or industrial scale preparation (solvent extracts), herbal plants have the potential to inhibit glucuronidation reaction. They may hinder endogenous substances such as bilirubin to be removed out from the body while permitting the entry of hazardous toxicants such as bisphenol A into the body. Most of the time, herbal products contain a plethora of phytochemical species which may cause beneficial or adversary effects. Identification of substances that are responsible for harmful side effects is an enigmatic task as plenty of phytochemicals exist in herbal products. As herbal supplement products are now exempted from FDA clinical scrutiny, productions by industries are more prone to adulteration and contamination. Therefore, it is imperative for clinicians to educate the public on the consequences of herb–drug interactions in order for them to gain vital knowledge regarding this issue, thus enabling them to wisely select the best treatment for their health-care need.

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## Conflicts of interest

There are no conflicts of interest.

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