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ORIGINAL ARTICLE

Inhibition of Human Cytochrome P450 2C8-catalyzed Amodiaquine N-desethylation: Effect of Five Traditionally and Commonly Used Herbs

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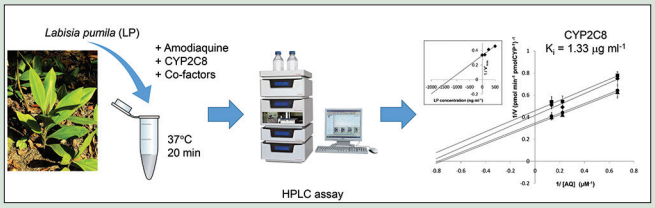
ABSTRACT

Background: In Southeast Asia and many parts of the world, herbal products are increasingly used in parallel with modern medicine. **Objective:** This study aimed to investigate the effects of herbs commonly used in Southeast Asia on activity of cytochrome P450 2C8 (CYP2C8), an important human hepatic enzyme in drug metabolism. **Materials and Methods:** The selected herbs, such as *Eurycoma longifolia Jack* (ELJ), *Labisia pumila* (LP), *Echinacea purpurea* (EP), *Andrographis paniculata* (AP), and *Ginkgo biloba* (GB), were subjected to inhibition studies using an *in vitro* CYP2C8 activity marker, amodiaquine N-desethylase assay. Inhibition parameters, inhibitory concentration 50% (IC_{50}), and K_i values were determined to study the potency and mode of inhibition. **Results:** All herbs inhibited CYP2C8 with the following order of potency: LP > ELJ > GB > AP > EP. LP and ELJ inhibited potently at K_i's of 2 and 4 times the K_i of quercetin, the positive control. The inhibition by LP was uncompetitive in nature as compared to competitive or mixed type inhibition observed with other herbs. GB exhibited moderate inhibitory effect at a K_i 6 times larger than quercetin K_i. AP and EP, on the other hand, showed only weak inhibition. **Conclusion:** The herbs we chose represented the more commonly used herbs in Southeast Asia where collision of tradition and modernization in healthcare, if not properly managed, may lead to therapeutic misadventures. We conclude that concurrent consumption of some herbs, in particular, LP and ELJ, may have relevance in drug-herb interactions via CYP2C8 inhibition *in vivo*.

Key words: Cytochrome P450, cytochrome P450 2C8, drug-herb interaction, inhibition

SUMMARY

- Herbs are increasingly used in parallel with modern medicines nowadays. In this study five commonly used herbs in Southeast Asia region, ELJ, LP, EP, AP and GB, were investigated for their *in vitro* inhibitory potency on CYP2C8, an important drug-metaboliz-ing human hepatic enzyme. All herbs inhibited CYP2C8 activity marker, amodiaquine N-desethylation, with potency order of LP > ELJ > GB >AP > EP. LP, ELJ and GB exhibited K_i values of 2, 4 and 6 times the K_i of quercetin, the positive control, indicating potent to moderate degree of enzyme inhibition. AP and EP, on the other hand, showed only weak inhibition. In summary, concurrent consumption of some herbs especially LP and ELJ may have relevance in drug-herb interactions via CYP2C8 inhibition *in vivo*.



Abbreviations Used: AQ: Amodiaquine, AP: *Andrographis paniculata*, CYP: Cytochrome P450, DEAQ: Desethylamodiaquine, EP: *Echinacea purpurea*, ELJ: *Eurycoma longifolia Jack*, GB: *Ginkgo biloba*, K_i: Inhibition constant, LP: *Labisia pumila*, Vmax: Maximal velocity, K_m: Michaelis-Menten constant.

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INTRODUCTION

Traditional preparations including herbs and "natural food" are widely used for preventive and therapeutic purposes. Societies have strong and long-standing beliefs that they are effective and safe. Some flavonoids in plants and food products are however able to induce or inhibit cytochrome P450 (CYP) enzymes in humans and animals.[1,2] Little is however known about these interactions, and patients and physicians may actually be unsuspecting of the potential interactions. Many physicians are also unaware that their patients are consuming traditional preparations, thus further potentially allowing for undetected adverse drug-herb interactions. However, increased toxicity of drugs has been attributed to inhibition of CYP enzymes by herbs.[3,4]

We chose cytochrome P450 2C8 (CYP2C8) in our study. Contribution of CYP2C8 to drug elimination in humans has been underestimated; its role however is beginning to be firmly established in recent years. This enzyme is primarily responsible for the metabolism of cerivastatin, paclitaxel, antidiabetic agents such as rosiglitazone and troglitazone, and antimalarial drugs amodiaquine (AQ) and chloroquine.[5] There also appears to be a degree of overlapping of substrate specificity between CYP2C8 and CYP3A4. For example, CYP2C8 contributes in part to the metabolism of the predominantly CYP3A4 substrates carbamazepine, verapamil, and zopiclone. Conversely, CYP3A4 contributes in part to cerivastatin and paclitaxel metabolism[5] mediated by CYP2C8. CYP2C8

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has also been implicated in the metabolism of endogenous compounds such as the oxidation of retinoids and fatty acids, including all-trans retinoic acid and arachidonic acid,[6,7] suggesting potential roles in the pathophysiology of diseases related to inflammatory processes. To the best of our knowledge, very little study has been carried out to investigate interaction between herbs and CYP2C8. We have therefore investigated the *in vitro* inhibitory effects of *Eurycoma longifolia Jack* (ELJ), *Labisia pumila* (LP), *Andrographis paniculata* (AP), *Echinacea purpurea* (EP), and *Ginkgo biloba* (GB), some of the popular herbs used in Malaysia and neighboring countries on CYP2C8 catalytic activity. This is a part of our continuing efforts to define potential drug-herb interactions of relevance in Southeast Asia. ELJ is a flowering plant in the Simaroubaceae family and is locally known as "Tongkat Ali" (literally means "Ali's walking stick"). The roots are used for dysentery, fever, persistent fever, malaria, syphilis, smallpox, and sexual insufficiency.[8-10] The notoriety of ELJ stems from its widespread use as an aphrodisiac. There are reports which have demonstrated that mice treated with ELJ regularly were found to be sexually hyperactive and had a higher sperm count.[11,12] The plant contains quassinoids, the biologically active components of the plant, which give a bitter taste for the herb.[10] LP is locally known as "Kacip Fatimah." It is from the family *Myrsinaceae*. It is rich in phytoestrogen, isoflavones, and antioxidants and is traditionally used for menopausal symptoms, menstrual irregularities, and in postpartum care. LP also contains a benzoquinone derivative and a mixture of resorcinol and pelargonidin derivatives.[13] AP is from the family *Acanthacea*. In general, it is known as "Hempedu bumi" that literally translates as the "bile of earth" due to its bitter taste. AP has traditionally been used to treat infections and some other diseases. AP contains andrographolide, a bitter bicyclic diterpenoid lactone. It has also been shown to have protozoacidal activity, inhibitory activity on platelet aggregation, immunostimulatory activity, anticancer activity, and antidiabetic activity.[14-16] While ELJ, LP, and AP are herbs of local origin in Southeast Asia, EP and GB are imported herbs in this region. EP from family *Compositae* is native to Midwestern North America. It has become the leading item in the current herbal renaissance in Southeast Asia and may parts of the world. EP contains phenol compounds such as cichoric and caftaric, alkylamides, and polysaccharides which have demonstrable immunomodulatory effects *in vitro*.[17] Similar to EP, GB is also another commonly used herb worldwide. GB from the family *Ginkgoaceae* is native to China and has been used in traditional Chinese medicine for centuries to treat circulatory disorders and to enhance memory.[18] GB contains ginkgo flavone glycoside and terpene lactones (ginkgolides, bilobalide). It has been suggested that its pharmacological effects are due to its antioxidant ability to scavenge free radicals.[18,19]

Given the widespread use of the above mentioned herbs in this region, coupled with the likelihood that these herbal medications would be given together with modern drugs, it is important to determine whether they can alter the disposition and effects of coadministered drugs in humans. The present study was undertaken to investigate the inhibitory effect of the herbs on CYP2C8 activity using AQ N-desethylase assay as activity marker. Inhibitory concentration 50% (IC_{50}) and K_i values were measured to determine both mode and potency of the inhibition observed.

MATERIALS AND METHODS

Natural products from medicinal plant and food

ELJ, LP, AP, EP, and GB aqueous extracts in powder form were obtained from the herbal depository of Professor Dr. Siti Amrah Sulaiman, Department of Pharmacology, Universiti Sains Malaysia in which the voucher specimens were deposited. The plants were collected from the Malaysian forest and botanic gardens in various locations in the states of Penang and Kelantan, and authenticated before extraction. The extracts were readily soluble in water and had been routinely prepared in the laboratory using a standard procedure. Briefly, dried raw material of the herbal plants was grounded, soaked, and extracted in distilled water. The homogenized suspensions were then heated at 45–60°C in water bath and filtered through a Whatman No. 1 filter paper. The volume of the extracts was then reduced by evaporation and later spray-dried in the Buchii Spray Drier to make the aqueous extracts. All the extracts were kept at 4°C until further use.

Drugs, chemicals, and reagents

AQ and desethylamodiaquine (DEAQ) were generously provided by Dr. Collen Masimirembwa, of the African Institute of Biotechnology, Harare, Zimbabwe. All other chemicals, reagents, and solvents were of the highest analytical grade available and purchased from various commercial sources.

Expression of cytochrome P450 2C8 in *Escherichia coli*

Human CYP2C8 (pCW-CYP2C8 17α) and NADPH CYP reductase (pACYC-OmpA/OxR) (donations from Professors John Miners and Donald Birkett, Flinders University, Adelaide, Australia) were cotransformed into *Escherichia coli* DH5α bacterial cells. The recombinant proteins were expressed and harvested according to method modified from published protocol.[20]

In vitro enzymatic incubation assay

The basic incubation assay contained 3 pmol of expressed CYP2C8 per incubation, 3.3 mM $MgCl_2$, 1 mM NADP, 3.3 mM glucose 6-phosphate, 1 U/ml glucose-6-phosphate dehydrogenase, 100 mM potassium phosphate buffer (pH 7.4), and AQ (0.5–70 μM), in a final volume of 1 ml. AQ was first dried under a stream of nitrogen before being vortex-dissolved in phosphate buffer before addition of the other reagents. The reaction was initiated by adding NADPH-generating system after a preincubation period of 5 min at 37°C. This was followed by a further incubation for 20 min. The incubation was terminated by the addition of 500 μl ice-cold acetonitrile after which the tubes were placed immediately on ice. The mixture was then centrifuged at 13,000 g for 5 min. The supernatant was transferred to a V-tube and dried under a stream of nitrogen. The residue was reconstituted in 125 μl of mobile phase for injection onto the high-performance liquid chromatography (HPLC).

High-performance liquid chromatography assay

We modified the HPLC method by Li *et al.*,[21] which was used to separate and quantify AQ and DEAQ in the incubation mixtures. The HPLC system consisted of Gilson 307 pumps, Gilson 811 B dynamic mixer, and Gilson auto-injector 234 connected to 100 μl injection loop. Detection was done using Gilson 115 variable ultraviolet detector set at 230 nm. Separation was achieved using a mobile phase consisting of 10% acetonitrile in 90% ammonium acetate (0.01 M), pH3, flowing through an Inertsil® C8-3 column (4.6 mm × 250 mm i.d., 5 μm particle size) (GL Sciences, Inc., Torrance, CA, USA) at a flow rate of 1 ml/min. Data were analyzed using an IBM® compatible computer loaded with Unipoint® software (version 2.00, Gilson Company, Inc., Lewis Center, OH, USA). AQ and DEAQ were resolved to baseline with retention times of 18.8 min and 13.5 min, respectively. Our calibration curve was linear within 20–500 ng/ml concentration range. Our inter- and intra-day variations were within levels which were recommended by the Food and Drug Administration under the optimum analytical method used.[21]

Michaelis–Menten kinetics and inhibition studies

The Michaelis–Menten kinetics of AQ *N*-desethylation by CYP2C8 was determined using eight substrate concentrations between 0.5 and 70 μM. For inhibition studies, the herbal extracts were dissolved in water. Quercetin, the positive control for CYP2C8, was dissolved in methanol. The extracts and the control were added to the *in vitro* incubation assay mixtures to screen for inhibitory effects. The organic solvent added as solvent for quercetin (methanol) did not exceed 1% v/v in the final incubation mixture. AQ *N*-desethylase activity was measured at various inhibitor concentrations (0 ng/ml to 1500 ng/ml for herbal extracts and 0–10 μM for quercetin) at each of three substrate concentrations (approximately K_m, 3 × K_m, and 5 × K_m). Substrate and inhibitor solutions were dried under a stream of nitrogen before vortex-mixing in phosphate buffer and added into the reactions tubes while on ice. After a 5 min preincubation at 37°C, NADPH-generating system was added to initiate the reaction. Kinetic studies were undertaken to determine the mechanism of inhibition and to calculate the apparent IC_{50} and inhibition constant (K_i).

Data analysis

Kinetic parameters of N-DEAQ formation (V_{max} and K_m) and inhibition data were determined by nonlinear least-squares regression analysis using the Enzyme Kinetics module of SigmaPlot version 8.0 (SPSS, Inc., Chicago, IL, USA). These parameters were initially estimated from the best fit line using least-squares linear regression of inverse substrate concentration versus inverse velocity (Lineweaver–Burk) plots. These estimates were subsequently used in analyses using the SigmaPlot program. The mechanism of inhibition was determined graphically from the Lineweaver–Burk plots and enzyme inhibition models analyzed through the Enzyme Kinetics module. Inhibition data from experiments that were conducted using multiple compound concentrations were represented by Lineweaver–Burk and secondary plots. IC_{50} (concentration of inhibitor causing 50% inhibition of original enzyme activity) values were also determined using nonlinear regression analysis as mentioned above.

RESULTS

Enzymatic kinetic parameters for cytochrome P450 2C8-mediated N-desethylation

The K_m and V_{max} values for N-desethylation by the expressed CYP2C8 were 1.28 ± 0.05 μM and 2.41 ± 0.02 pmol/min/pmol of CYP2C8 protein, respectively. The results were comparable to the values of studies by Li *et al.*[21] Similarly, screening of the assay with quercetin, the positive control, revealed IC_{50} and K_i values of 4.20 ± 0.46 μM (1.27 ± 0.14 μg/ml) and 2.07 ± 0.23 μM (0.63 ± 0.07 μg/ml) and a competitive mode [Figure 1] in inhibition, similar to those reported in literature.[22] This has indicated that the assay being used was operating appropriately and served as a valid activity marker for CYP2C8.

Inhibitory concentration 50% values of cytochrome P450 2C8 inhibition

The IC_{50} values of ELJ, LP, AP, GB, and EP were determined across various inhibitor concentrations using nonlinear regression analysis. As shown in Table 1, the herbs showed differential inhibition toward AQ N-desethylation with a relative order of inhibitory potency of LP > ELJ > GB > AP > EP. Potent to moderate inhibition was noticed in LP, ELJ, and GB with IC_{50} ranging from 2.62 to 6.65 μg/ml. These IC_{50} values were within the same order of magnitude with quercetin IC_{50}. In contrast, only mild to weak inhibition was observed for AP and EP (IC_{50} above 10 μg/ml).

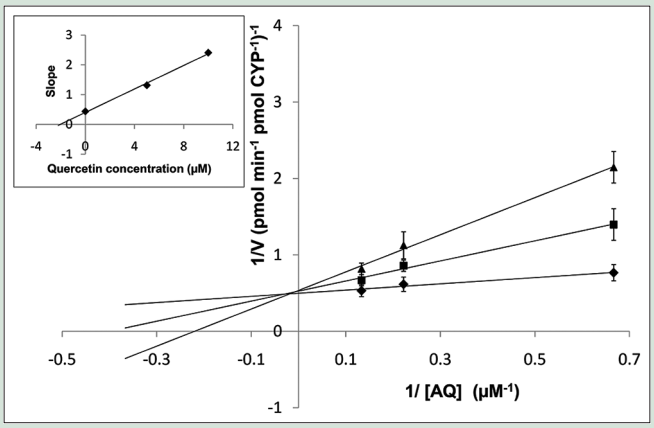

Figure 1: Primary Lineweaver–Burk plot and secondary plot (inset, slope of Lineweaver–Burk plot vs. inhibitor concentration) for the inhibition of cytochrome P450 2C8-mediated amodiaquine N-desethylation by quercetin. Reactions were performed in the presence of amodiaquine at 3 different concentrations (1.5 μM, 4.5 μM, and 7.5 μM) at 0 μM (♦), 5 μM (■), and 10 μM (▲) of quercetin in the presence of cytochrome P450 2C8 recombinant protein (3 pmol/ml) and NADPH system in 100 mM phosphate buffer (pH 7.4), at a final volume of 1 ml at 37°C for 20 min. Each point represents the mean of separate experiments performed in triplicates

Table 1: Inhibition potencies and modes of various herbs on cytochrome P450 2C8-mediated amodiaquine N-desethylation

Inhibitor	Inhibition mode	K_i (μg/ml)[a]	IC_{50} (μg/ml)[a]
Eurycoma longifolia	Competitive	2.41±0.59	5.79±1.17
Labisia pumila	Uncompetitive	1.33±0.33	2.62±0.66
Andrographis paniculata	Mixed type	6.26±1.37	14.80±2.74
Ginkgo biloba	Mixed type	3.50±1.17	6.65±1.94
Echinacea purpurea	Mixed type	19.20±5.10	72.80±10.20
Quercetin	Competitive	0.63±0.07 (2.07±0.23 μM)	1.27±0.14 (4.20±0.46 μM)

[a]Each value represents the mean±SD of three independent experiments.
SD: Standard deviation; IC_{50}: Inhibitory concentration 50%

Cytochrome P450 2C8 inhibition kinetic analysis

To further characterize the inhibition of CYP2C8 activity, enzyme inhibition kinetic analysis was performed using several substrate and inhibitor concentrations. Results obtained were used to produce the Lineweaver–Burk double-reciprocal plots indicating the possible inhibition modes for each reaction. The apparent K_i values were later estimated using the secondary plots of the slope or ordinate intercept of the Lineweaver–Burk plots against individual inhibitor concentrations. These initial kinetics estimates were subsequently used to determine K_i values using nonlinear regression analysis. As illustrated in Table 1, the K_i followed the same rank order of potency as seen in the IC_{50} values determined earlier with LP displaying the most potent inhibition and EP the mildest effect.

Inhibition by LP obeyed the uncompetitive inhibition model with parallel alterations in V_{max} and K_m values across different inhibitor concentrations. This was represented by the parallel lines, each with its different intercepts on both axes of the Lineweaver–Burk plot [Figure 2]. The K_i value was determined to be 1.33 ± 0.33 μg/ml. Inhibition by ELJ was best described by competitive inhibition model showing a K_i of 2.41 ± 0.59 μg/ml. The K_i values of both LP and ELJ were both close to K_i of quercetin (only 2 and 4 times larger in value), indicating potent inhibitory effect of the two herbs. GB ranked third

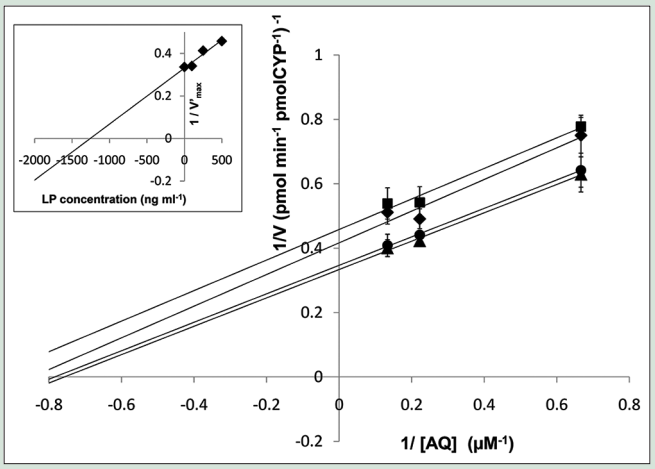

Figure 2: Primary Lineweaver–Burk plot and secondary plot (inset, $1/V'_{max}$ vs. inhibitor concentration) for the inhibition of cytochrome P450 2C8-mediated amodiaquine N-desethylation by *Labisia pumila* extract. Reactions were performed in the presence of amodiaquine at three different concentrations (1.5 μM, 4.5 μM, and 7.5 μM) at 0 ng/ml (▲),100 ng/ml (●), 250 ng/ml (♦), and 500 ng/ml (■) of the extract in the presence of cytochrome P450 2C8 recombinant protein (3 pmol/ml) and NADPH system in 100 mM phosphate buffer (pH 7.4), at a final volume of 1 ml at 37°C for 20 min. Each point represents the mean of separate experiments performed in triplicates

in inhibitory potency against CYP2C8 with a mixed type inhibition and a K_i of 3.50 ± 1.17 μg/ml. Inhibitions by AP and EP were also of mixed-type and weaker with larger K_i values (10 times or more larger than quercetin's K_i).

DISCUSSION

Malaysia is a country rich in traditions and tropical flora and fauna. Earliest precolonial medical care was confined to traditional remedies which were practiced among local populations of Malays, Chinese, and other ethnic groups. Healthcare in Malaysia has however undergone radical transformations. The advent of colonialism brought Western medical practice into the country. Since the country's independence in August 1957, the system of medical care transferred from the British colonial rule has been transformed to meet the needs of emerging diseases, as well as national political requirements. Scenarios in many neighboring countries in Southeast Asia share many similarities with regard to incorporation of herbal therapy into the mainstream healthcare system and the combined use of traditional remedies and modern drugs as major treatment modality for chronic diseases. This collision of tradition and modernization in healthcare, if not properly managed, can lead to therapeutic misadventures when patients combine traditional and modern medicines.

Five commonly used herbs in the region, namely ELJ, LP, AP, GB, and EP, were selected for investigation as they represent the most commonly used herbal remedies by local populations. A large number of products from these herbs either supplied as crude extracts or in commercially available dosage forms are made available in the market. These easily accessed products have made drug-herb interaction; a potential safety issue as CYP-herb interaction has become an important determinant of untoward drug adverse effects. We investigated CYP2C8 as this isoform is involved in metabolism of therapeutically important drugs including paclitaxel, cerivastatin, AQ, and troglitazone, many of which are highly potent and toxic therapeutic agents. The aqueous extracts of the five herbs were studied because all these herbs are normally administered as infusion from various plant parts in water. For those available in pharmaceutical dosage forms, the preparations are mainly in the form of purified extraction powder incorporated in tablet or encapsulated whereby the powder derived from extraction using water or water-miscible solvent such as ethanol is used. Therefore, aqueous extracts represent the most common form of administration in patients either as herbal preparations or pharmaceutical dose forms of these herbs.

Of the five herbs, LP appears to be the most potent inhibitor for CYP2C8. Its inhibition was consistent with uncompetitive inhibition with a K_i of 1.33 ± 0.33 μg/ml. The IC_{50} value was twice the value of IC_{50}, i.e., 2.62 ± 0.66 μg/ml. It is interesting that the herb exhibited uncompetitive inhibition of CYP2C8 activity. Uncompetitive inhibition is rare for most enzymes. The inhibitor components (I) bind only to the enzyme-substrate (ES) complex and, therefore, have very specific effect. As the ES-inhibitor (ESI) complex is nonproductive, high inhibitor concentration can reduce greatly the reaction velocity. Therefore, the uncompetitive inhibition property of LP may result in fluctuating inhibition activity as it is dependent on the amount of the ES formed in our body, as well as LP and CYP2C8 substrate concentrations. It would be expected that LP inhibitor(s) would exert greater inhibitory effect at higher substrate concentration as more ES complex would be formed hence greater amount of ESI complex. The herb demands further investigation to identify the chemical properties of the component(s) that may be responsible for the inhibition. In term of active constituents, LP is known to be rich in phytoestrogen, isoflavones, benzoquinone derivative, resorcinol, and pelargonidin derivatives.[23,24] Many of these constituents may serve as potential CYP2C8 inhibitor. Phytoestrogens, one of the major constituents, share structural similarity to natural estrogen and thus have the potential to modulate CYP2C8 activity. Many synthetic hormonal agents including estrogens and progesterones are known to potently inhibit CYP activities.[25,26] Similarly, isoflavones such as daidzein and genistein which have been reported in LP are known inhibitor for CYP.[1,27] The potent inhibitory effect on CYP2C8, here, may warrant confirmatory studies in animals and human subjects. This is of clinical relevance as the herb is commonly used in female patients for menstrual, menopausal, and postpartum conditions who are likely to also take synthetic hormonal agents such as estrogens and progesterones as part of their hormone replacement therapy. Concurrent use of LP may thus cause elevated level of these agents hence side effect or toxicity.

ELJ ranked second as potent CYP2C8 inhibitor after LP. Its inhibition obeyed a competitive inhibition model. The K_i obtained was 2.41 ± 0.59 μg/ml and the IC_{50} was 5.79 ± 1.17 μg/ml. Lineweaver–Burk plot profile and IC_{50} of twice the value of K_i confirmed that ELJ extract has a competitive inhibition effect on CYP2C8 activity. This relatively potent inhibition of ELJ may be a cause for safety concern in patients. Of interest, here is that ELJ is popularly consumed for it aphrodisiac effect, hence consumers of the herbs are normally middle-aged and elderly men who take ELJ in the hope of enhancing their sexuality. They are also the subset of population commonly afflicted with other chronic illnesses such as hypertension and diabetes mellitus. Moreover, the problem is confounded by the availability of ELJ in various commercial herbal products and beverage drinks on local market in this region. ELJ in powder form has been incorporated in different pharmaceutical dose forms (e.g., capsules, tablets) as well as favorite drinks such as tea and coffee. Concurrent intake of ethical drugs with ELJ either as herbal supplement or favorite drink by patients may be of concern due to drug-herb interaction. Results of this study suggest that these ELJ preparations should be examined for potential pharmacokinetic drug interactions *in vivo*.

GB demonstrated relatively potent mixed type inhibition and ranked third after LP and ELJ. Numerous studies on GB-CYP interaction have been carried out. Budzinski *et al.* and Umegaki *et al.* reported that commercial GB extract significantly inhibited human CYP3A4 and rat CYP3A activities *in vitro*, respectively,[28,29] whereas the feeding of GB for 4 weeks markedly induced CYP3A1 and CYP3A2 mRNA expression, weakening the hypotensive effect of nicardipine in rats.[30] Another study showed that the concomitant use of GB in rats increased the bioavailability of diltiazem by inhibiting both intestinal and hepatic metabolism, at least in part, via a mechanism-based inhibition for CYP3A.[31] A more recent study[32] on CYP2C9 demonstrated that GB exhibited *in vitro* inhibition of S-warfarin oxidation with a K_i of 9.1 μg/ml, but this was not observed *in vivo*. This result is of relevance to our study because CYP2C9 is a closely related isoform to CYP2C8 and its determined K_i is close to our CYP2C8's K_i (i.e., 3.50 μg/ml). The fact that this low K_i value for CYP2C9 was inconsistent with *in vivo* observation (negligible inhibition) highlights the importance of carrying confirmatory study to prove that GB is, in fact, a potent *in vivo* CYP2C8 inhibitor.

AP displayed moderate IC_{50} and K_i of 14.80 ± 2.74 and 6.26 ± 1.37 μg/ml, respectively. *In vitro* interaction of AP has been reported with many CYP isoforms. Differential inhibition pattern was observed with this herb whereby CYP1A2, CYP2C9, and CYP3A4 were found to be inhibited more than CYP2D6, CYP2E1, and CYP2B isoforms.[33-36] To the best of our knowledge, this is the first report of CYP2C8 inhibition by AP. The moderate degree of inhibition seen in this study however indicates that clinically significant interactions between AP and CYP2C8 substrates are unlikely. As for EP, the extracts of the herb have been found, *in vitro*, to affect the activity of CYP3A4.[37] It has also been found to cause inhibition of CYP1A2 and intestinal CYP3A activity and to induce hepatic CYP3A activity.[38] In our study, EP was however found to display only moderate to weak inhibition activity against AQ desethylation in CYP2C8; hence, the chances for any significant CYP2C8-EP interaction would be remote.

CONCLUSION

The herbs we chose in this study represent the more commonly used and popular herbal products that are widely available through formal (clinics and pharmacies) and informal sources (retail outlets and traditional medicine practitioners), taken by many for health maintenance and treatments of common ailments. Our study revealed that all the herbs tested showed differential inhibition of CYP2C8. Relatively potent inhibition was observed for LP, ELJ, and GB whereas the effect seen with AP and EP was mild. These results suggest that the use of high amounts of LP, ELJ, and GB herbal preparations may cause an interaction with drugs metabolized by CYP2C8 in some individuals. It is important to note, however, that these *in vitro* inhibitions do not necessarily translate into drug interactions in clinical situations. *In vivo* studies on the interactions between these herbs and CYP2C8 selective probes are required to determine the clinical relevance of CYP2C8 inhibition.

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Conflicts of interest

There are no conflicts of interest.

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