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ORIGINAL ARTICLE

Altered Cytochrome P450 Profiles by *Plumbago indica* Linn. and Plumbagin after Oral Administration in Mice

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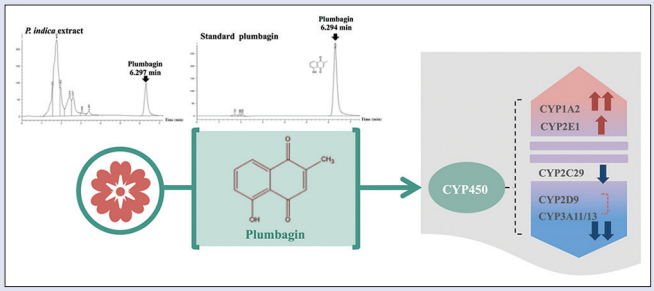
ABSTRACT

Background: *Plumbago indica* Linn. and its active constituent, plumbagin, are conventionally used in Thai alternative medicines, but information regarding their effects on cytochrome P450 (CYP450) enzymes is limited. **Objective:** To establish the effects of *P. indica* Linn. and plumbagin on CYP450 profiles. **Materials and Methods:** Adult male mice were orally administered *P. indica* extract (20, 200, and 1000 mg/kg/day) or plumbagin (1, 5, and 15 mg/kg/day) for 14 days. The levels of hepatic CYP450 mRNA and protein were assessed using reverse transcription/real-time polymerase chain reaction and immunoblotting, respectively, and specific enzyme reactions were performed to determine the enzyme activities. **Results:** Expression of *Cyp1a2* was induced by both *P. indica* and plumbagin, while *P. indica*, but not plumbagin, slightly suppressed *Cyp2c29* expression. Expression of *Cyp2d9* was suppressed by both *P. indica* and plumbagin. Expression of *Cyp2e1* was unchanged, but *P. indica* at the lowest dose increased *Cyp2e1* activity. *P. indica* and plumbagin dose-dependently suppressed the expression of *Cyp3a11*/13 and its activity. **Conclusions:** Modulation of CYP450 profiles by *P. indica* and plumbagin is a concern as there are risks of drug–herb interaction.

Key words: *Cyp1a2, Cyp2c29, Cyp2d9, Cyp2e1, Cyp3a11*/13

SUMMARY

- Modulation of cytochrome P450 profiles, induction of *Cyp1a2* and *Cyp2e1*, while suppression of *Cyp2d9* and *Cyp3a11*/13, by *Plumbago indica* and plumbagin are the concerns as there are risks of hepatotoxicity and drug–herb interaction, especially a use at high quantity or for a long period.



Abbreviations used: CYP450: Cytochrome P450; BSA: Bovine serum albumin; GAPDH; Glyceraldehyde 3-phosphate dehydrogenase.

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INTRODUCTION

To date, the use of herbal plants as alternative medicine has become increasingly common worldwide.[1-3] Recent evidence showed that herbal supplements have been regularly consumed by approximately 20% of the population,[4] leading to increased awareness in herb–drug interactions which can result in undesired adverse effects.[2]

Herb–drug interactions occur through several mechanisms. However, the most common mechanism involves modulation of pharmacokinetics of a concomitant drug by an herb,[1,3] which is an inducer/inhibitor of cytochrome P450 (CYP450), a major superfamily of hepatic metabolizing enzymes that biotransformed over 90% of clinical drugs.[5-7] Induction of the CYP450 enzymes results in an increase in metabolism and thus excretion of its substrate, while the inhibition leads to a decrease in excretion of the substrate and as a result prolongs elimination time.[3]

Plumbago indica Linn. (family *Plumbaginaceae*), a shrub widely distributed in tropical and subtropical regions of Africa, Australia, and Asia,[8] contains plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone), a yellowish quinonoid, as a major constituent that contributes to the medicinal properties.[8,9] The *P. indica* root has been used as an active ingredient in Ayurveda for diarrhea, indigestion, and several skin diseases and has been applied as an anthelmintic, appetite stimulant, and rubefacient agent.[8-10] The root of *P. indica*, the part mostly contained in the medicinal remedies, yields the highest amount of plumbagin (0.17% w/w) among *Plumbago* species.[11] *P. indica* extract possessed antibacterial[12-14] and antifungal activities.[15] *Plumbago* species have been claimed for antifertility property.[16] Particularly, *P. indica* was employed in ancient remedies for abortion.[10] Plumbagin possessed biological activities similar to *P. indica* extracts, i.e., anthelmintic,[17] antimalarial,[18] antibacterial,[13] antifungal,[19] anti-inflammatory,[20,21] antidiabetic,[22] immunosuppressive,[23] and anticancer activities,[20,24-26] and abortifacient.[16]

Despite their biological values and current uses, information regarding the effects of *P. indica* and plumbagin on CYP450s is still limited. To

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pursue the U.S. Food and Drug Administration recommendation to predict the CYP450-associated herb–drug interactions, this study aimed to examine how *P. indica* extract and plumbagin influence the expressions of *Cyp1a2*, *Cyp2c29*, *Cyp2e1*, and *Cyp3a11*/13 in the mouse livers. The observations revealed that the *P. indica* extract and plumbagin potentially modified the CYP450 profiles at molecular levels, namely mRNA and protein expression, including enzyme activities, subsequently leading to a risk of herb–drug interaction. Therefore, the concomitant use of a product containing *P. indica* or plumbagin with modern medication is of concern to justify the potential benefit and risk.

MATERIALS AND METHODS

Chemicals and reagents

Plumbagin was a product of LKT Laboratories (St. Paul, MN, USA). Acrylamide/bis-acrylamide 30% (29:1) was obtained from Bio-Rad (Hercules, CA, USA). Erythromycin, β-nicotinamide adenine dinucleotide phosphate reduced form (NADPH), bovine serum albumin (BSA), β-mercaptoethanol, *p*-nitrocatechol, and Tris base were supplied by Sigma-Aldrich Chemical (St. Louis, MO, USA). ReverTra Ace® was a product of Toyobo Co., Ltd. (Osaka, Japan). Taq DNA polymerase was purchased from Vivantis® (Malaysia). All other laboratory chemicals were of the highest purity from commercial suppliers.

Preparation of *Plumbago indica* extract and determination of plumbagin in the extract

The root of *P. indica* was bought from Mor Tong-In Thai Traditional Medicine (Mahasarakham, Thailand) in June 2014. The plant materials were identified by Dr. Waraporn Putalun, Faculty of Pharmaceutical Sciences, Khon Kaen University (Khon Kaen, Thailand), and the reference specimen (PANPB-PI 2014-002) was deposited at the Herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University. It was dried at 50°C in an oven and then shredded and extracted with methanol using a Soxhlet apparatus for 3 h before evaporated and freeze-dried into powder. %Yield of the extract was 33.40% by weight. Plumbagin content was analyzed on a Hypersil® ODS column (5 μm, 250 mm × 4.0 mm, Agilent Technologies, CA, USA) with an isocratic linear solvent system of 50% (v/v) acetonitrile at a flow rate of 1 mL/min using an Agilent 1260 Infinity high-performance liquid chromatography (HPLC) system (Agilent Technologies) coupled with a UV-Vis detector.[27] The chromatogram was monitored at the wavelength of 410 nm and analyzed with the ChemStation software (Agilent Technologies). Identification and quantification of plumbagin were performed based on retention time and peak area with the plumbagin authentic standard.

Animal treatments

Seven-week-old male ICR mice were obtained from the National Laboratory Animal Center, Mahidol University (Nakorn Pathom, Thailand). All mice were housed in an animal unit of Faculty of Pharmaceutical Sciences, Khon Kaen University. The animal handling and treatment protocol was approved by the Animal Ethics Committee for Use and Care of Khon Kaen University (Approval No. AEKKU11/2558). Mice were housed in polysulfone cages with wood-shaved bedding (5 per cage) under a controlled temperature (23°C ± 2°C) and humidity (45% ± 2%) with a 12-h dark/light cycle. Water and commercial rodent diet were supplied *ad libitum*. The mice ($n = 5$) were orally administered the *P. indica* extract (20, 100, and 1000 mg/kg/day) or plumbagin (1, 5, and 15 mg/kg/day) daily for 14 days. The control group was orally given the vehicle 0.5% carboxy methyl cellulose daily for the same period. At 24 h after the last treatments, the livers were collected and kept at −80°C for further analysis.

Quantitative determination of cytochrome P450 mRNA expressions using reverse transcription/real-time polymerase chain reaction

Guanidine thiocyanate-phenol-chloroform method was employed to prepare total RNA from the livers.[28] Total RNA was reversed transcribed to cDNA using ReverTra Ace® at 25°C for 10 min, 42°C for 60 min, and 95°C for 5 min, respectively. The reaction mixture for real-time polymerase chain reaction (PCR) contained 50 ng cDNA, 0.25 mM of each forward and reverse primers [Table 1], 1 mM dNTP mixture, 2 mM $MgCl_2$, 1× SYBR green I, and 1.25 unit of Taq DNA polymerase. Each PCR cycle was initiated at 95°C for 30 s, before lowering to the annealing temperature of each gene for 30 s, followed by the extension temperature at 72°C for 30 s. The mRNA levels were normalized to that of glyceraldehyde 3-phosphate dehydrogenase (*Gapdh*), a reference gene which was not affected by the treatments.[28]

Preparation of liver microsomes

Liver microsome was prepared as described previously.[29] In brief, the liver was homogenized in 1.15% KCl before centrifuged at 10,000 g for 10 min at 4°C, and then, the supernatant was subjected for ultracentrifugation at 104,000 g for 60 min at 4°C. The microsome pellet was reconstituted with ice-cold water and kept at −80°C before using. The protein content was determined by the method of Bradford using BSA as the standard.[29]

Table 1: Forward and reverse primer sequences

Gene	Accession number	Forward and reverse primers (5′ → 3′)	$T_{annealing}$ (°C)	Product size (bp)
Cyp1a2	NM_009993.3	F-CGTCAGCAAGCTTCAGAAGG R-ACGATGTTCAGCATCTCCTCG	57.0	144
Cyp2c29	NM_007815	F-ATCTGGTCGTGTTCCTAGCG R-AGTAGGCTTTGAGCCCAAATAC	50.0	218
Cyp2d9	NM_010006.2	F-ATTCTTGTTGCCCCCTCTCC R-TGGCAGGAAACTGCCCTACA	60.4	347
Cyp2e1	NM_021282.2	F-TCCCTAAGTATCCTCCGTGA R-GTAATCGAAGCGTTTGTTGA	50.0	529
Cyp3a11	NM_007818.3	F-TTTGGTAAAGTACTTGAGGCAGA R-CTGGGTTGTTGAGGGAATC	64.0	134
Cyp3a13	NM_007819.4	F-TGTGCTGGCTATCACAGATCC R-AAATACCCACTGGACCAAAGC	55.0	101
Gapdh	NM_008084.3	F-CCTCGTCCCGTAGACAAAATG R-TGAAGGGGTCGTTGATGGC	57.4	152

Determination of cytochrome P450 protein expressions using Western blotting

The expressions of CYP450 proteins were determined using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) coupled with immunoblotting[28,29] with some modifications. Briefly, each microsome sample (10 μg) was resolved on a 12% SDS-PAGE before transferred to a Hybond-C nitrocellulose membrane. The specific CYP450 proteins was detected using polyclonal antibody against *Cyp1a2* (Daiichi #299124), *Cyp2c9* (Abcam #ab4236), *Cyp2d9* (Santa Cruz #sc-23690), or *Cyp3a11*/13 (Daiichi #299223). *Gapdh* was employed as the reference gene and detected using polyclonal anti-*Gapdh* immunoglobulin G (IgG) (Abcam #EPR16891/ab181602). Then, the immunoblots were incubated with biotinylated anti-IgG, and the antigen-antibody complex was visualized using the 3,3'-diaminobenzidine peroxidase substrate kit.

Assessment of *p*-nitrophenol hydroxylation

Production of *p*-nitrocatechol from *p*-nitrophenol hydroxylation was assessed for *Cyp2e1* activity[30] with some modifications. Briefly, a reaction mixture of 150 μg microsomes, 1 mM ascorbic acid, 500 μM *p*-nitrophenol, 10 mM NADPH, and 0.1 M phosphate buffered saline (pH 7.0), in a total volume of 145 mL, was incubated at 37°C for 20 min before adding 0.6 N $HClO_4$. Then, the mixture was centrifuged at 1900 rpm for 15 min. The supernatant was collected and mixed with 10 N NaOH. The absorbance was measured at a wavelength of 490 nm compared with the *p*-nitrocatechol standard.

Assessment of erythromycin *N*-demethylase activity

Formaldehyde formation from erythromycin *N*-demethylation in the Hantzsch reaction was performed to assess CYP3A activity[31] with some modifications. Briefly, the reaction mixture of 1 mM NADPH, 15 mM $MgC1_2$, 1 mM erythromycin, 70 mM phosphate buffer (pH 7.4), and 15 mg microsomes, in a final volume of 100 mL, was incubated with NADPH to initiate the reaction at 37°C for 20 min. And then, the reaction was stopped by adding 12.5% trichloroacetic acid before centrifuged at 1900 rpm for 15 min. The supernatant was collected and mixed with the Nash reagent before incubated at 60°C for 15 min. The absorbance was measured at a wavelength of 405 nm compared with the formaldehyde standard.

Statistical analysis

The data were analyzed using one-way ANOVA followed by the least significant difference *post hoc* test (IBM SPSS statistics version 19, Armonk, New York, United States). $P < 0.05$ was considered statistically significant.

RESULTS

Contents of plumbagin in the *Plumbago indica* extract

The *P. indica* extract was quantitatively determined plumbagin content by the HPLC method.[27] The chromatograms of the *P. indica* extract and standard plumbagin are shown in Figure 1. The retention time of plumbagin was about 6.3 min. The content of plumbagin in the extract was 0.15 ± 0.003%.

Effects of the *Plumbago indica* extract and plumbagin on the profiles of cytochrome P450

The expression of *Cyp1a2* mRNA was significantly elevated by both *P. indica* and plumbagin [Figure 2a]. Correspondingly, the expression

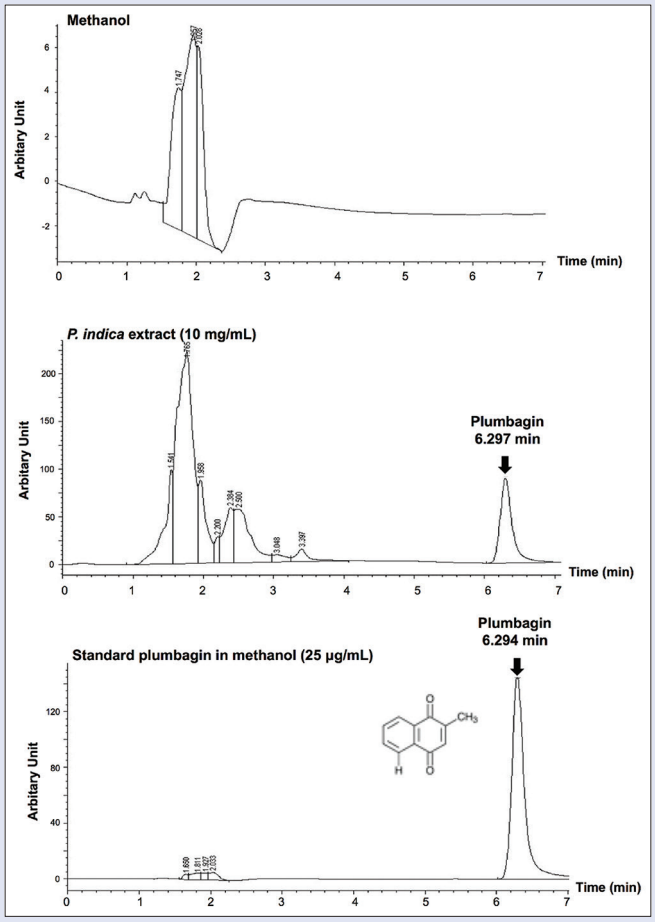

Figure 1: Chromatograms of the *Plumbago indica* extract and the plumbagin standard. (a) Methanol, (b) the *Plumbago indica* extract at a concentration of 10 mg/mL, and (c) the plumbagin standard at a concentration of 25 μg/mL

of CYP1A2 protein was persistently upregulated after the treatments of *P. indica* and plumbagin [Figure 2b]. By contrast, plumbagin did not significantly change the expression level of *Cyp2c29* mRNA [Figure 2a] while the *P. indica* extract at the two higher doses (200 and 1000 mg/kg/day) slightly suppressed those of *Cyp2c29* mRNA. The expression of CYP2C29 protein was correlated with those of *Cyp2c29* mRNA, in which the expression levels of CYP2C29 protein were significantly declined by the two higher doses of *P. indica* extract while the others were remained unchanged [Figure 2b]. The pattern of *Cyp2d9* mRNA expression tended to be dose-dependently suppressed [Figure 2a], whereas the expression of CYP2D9 protein was significantly downregulated by both of the *P. indica* extract and plumbagin [Figure 2b]. The expressions of *Cyp2e1* mRNA [Figure 2a] and *p*-nitrophenol hydroxylase responsible CYP2E1 activity [Figure 2c] were not significantly modified by either the *P. indica* extract or plumbagin, except the lowest dose of *P. indica* extract with a significant increase in the CYP2E1 activity. The expressions of *Cyp3a11* and *Cyp3a13* mRNAs were extensively suppressed in a dose-dependent pattern by both of the *P. indica* extract and plumbagin [Figure 2a], in accordance with the expression of CYP3A11/13 protein [Figure 2b]. Likewise, both the *P. indica* extract and plumbagin significantly inhibited the erythromycin *N*-demethylase responsible CYP3A activity [Figure 2c].

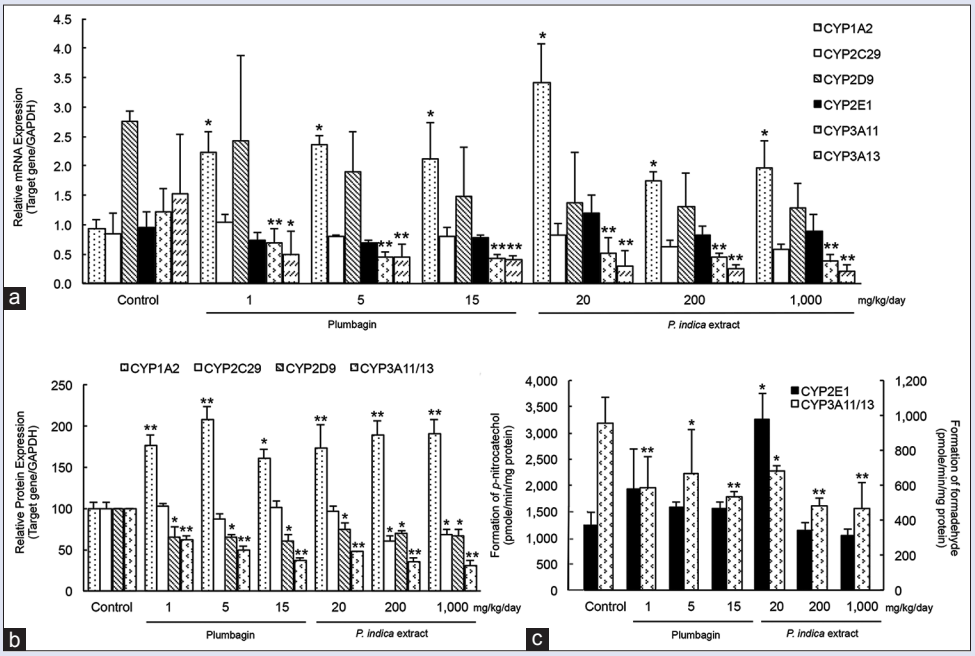

Figure 2: Effects of plumbagin and the *Plumbago indica* extract on the cytochrome P450 profiles. (a) Relative mRNA expression of cytochrome P450/glyceraldehyde 3-phosphate dehydrogenase, (b) relative protein expression of cytochrome P450/glyceraldehyde 3-phosphate dehydrogenase, and (c) formation of *p*-nitrocatechol and formaldehyde. *$P < 0.05$, **$P < 0.01$ versus control

DISCUSSION

The plumbagin content in the *P. indica* extract was presently found at 0.15% ± 0.003% dry weight which well correlated with its content in the chloroform extract at 0.17%[11] and the ethanol extract at 0.20%.[32] A small variation of the plumbagin content among these reports might occur from cultivation of the plant and season of harvesting, including storage procedure, and the extraction method.

While herbal supplements are popularly consumed, the incidence of herb–drug interactions is increasingly reported spontaneously. The most common mechanism responsible for drug interactions involves pharmacokinetic modulation of the concomitant drug via the modification of drug metabolizing enzymes profiles, especially CYP450s.[1,3] The examples of CYP450 modulatory herbs were St. John's Wort, a potent inducer of *CYP3A4*, *CYP2E1*, and *CYP2C19*;[7] grapefruit juice and black pepper, a potent *CYP3A4* inhibitor;[2,33] and *Ginkgo biloba* and pomegranate juice, an inhibitor of *CYP3A4*.[34,35] In the current study, we evaluated how *P. indica* and plumbagin affect the profiles of CYP450s, including *Cyp1a2*, *Cyp2c29*, *Cyp2d9*, *Cyp2e1*, and *Cyp3a11*/13, in the mouse livers.

CYP1A consists of *CYP1A1* and *CYP1A2*.[36] *CYP1A2* is one of the major CYP450s in human (~13%–15%) highly abundant in the liver and responsible for metabolism and elimination of exogenous substances, clinically important drugs, such as clozapine, tacrine, tizanidine, and theophylline, some procarcinogens such as benzo[*a*] pyrene and aflatoxin B1, and several important endogenous compounds, including steroids and arachidonic acids.[37] *P. indica* and plumbagin significantly elevated the expression levels of *Cyp1a2* mRNA and protein in mice. The induction mechanism of *Cyp1a* associated with heterodimerization of a cytosolic receptor, aryl hydrocarbon receptor (AhR), and AhR nuclear translocator along with upstream-enhancer elements which transmitted the induction signal to the promoter, resulting in the transcription and translation processes.[36] Despite the increasing levels of *Cyp1a2* mRNA and protein, neither the *P. indica* extract nor plumbagin exhibited the significant changes in the methoxyresorufin *O*-demethylase responsible CYP1A2 activity (data not shown). These evidences noted that the increases in either *Cyp1a2* mRNA or protein were probably not enough to convey physiological effects. On the other hand, an *in vitro* study showed inhibitory effect of plumbagin on CYP1A2 activity in human microsomes.[38] These findings might be due to the differences in the assessment and limitation of the method, in which our study was an *in vivo* animal model while Sumsakul *et al.* employed human microsomes, an *in vitro* model.[38] In addition, it should be noted that an *in vitro* study could not demonstrate an inductive effect of CYP450.

CYP2C is a subfamily responsible for metabolism of 16% clinical drugs, e.g., (S)-mephenytoin, omeprazole, tricyclic antidepressants, proguanil, warfarin, non-steroidal antiinflammatory drugs (NSAIDs), tolbutamide, nelfinavir, paclitaxel, and carisoprodol,[5] and mostly found in the liver, followed by heart, and cardiac tissue.[36,39] Human *CYP2C* comprises *CYP2C8*, *CYP2C9*, *CYP2C18*, and *CYP2C19*, while mouse *Cyp2c* exists as much as 15 isoforms.[40] A study in amino acid sequence alignment showed that human *CYP2C9* was homologous to both mouse *Cyp2c29* and mouse *Cyp2c55*.[41] In this study, though *P. indica* and plumbagin did not significantly suppress the expression of *Cyp2c29* mRNA, the two higher doses of the *P. indica* extract showed the inhibitory effect on the expression of CYP2C29 protein. Correspondingly, a previous study showed that plumbagin inhibited human CYP2C19 activity at 35 folds greater than the selective inhibitor, nootkatone.[38]

CYP2D is classified as a noninducible isoform by xenobiotics.[42] CYP2D metabolizes 20%–50% clinical drugs.[43] Substrates of CYP2D are basic lipophilic nitrogen-containing molecules and alkaloids.[44] Although drug interactions due to *CYP2D* activation do not normally happen, epigenetic variations of CYP2D enzymes and inhibition of CYP2D enzyme activity can be occurred.[44,45] Of nine *Cyp2d* isoforms in mouse, *Cyp2d9* showed high amino acid identity to *CYP2D6*, a major human *CYP2D*.[40] In this study, the expressions of *Cyp2d9* mRNA and protein were dose-dependently inhibited by both of the *P. indica* extract and plumbagin. These observations suggested that plumbagin

and/or other constituents in the extract were metabolized by CYP2D9 and consequently resulted in a decrease in the *Cyp2d9* expression. Therefore, use of a product containing *P. indica* or plumbagin concomitant with a CYP2D substrate, i.e., neuroactive drugs such as tricyclic antidepressants, e.g., imipramine, amitriptyline, clomipramine, and desipramine, and selective serotonin reuptake inhibitors, e.g., fluoxetine and sertraline,[44] may cause unwanted effects due to slower rate of metabolism and thus excretion of these drugs.

CYP2E1 is a major CYP450 isoform that causes hepatic oxidative injury.[46-48] Human *CYP2E1* has been similar to mouse, rat, and rabbit *Cyp2e1*, making an *in vivo* study of CYP2E1 activity in an animal model reliably predicts the CYP2E1 activity in human.[49,50] CYP2E1 is responsible for catalyzing metabolism and bioactivation of low-molecular-weight molecules, i.e., procarcinogens, and drugs, and metabolism of endogenous fatty acids and ketones.[51] CYP2E1 is inducible via posttranscription and posttranslation pathways of protein stabilization and inhibition of ubiquitin-mediated protein degradation.[52,53] Stabilization of mRNA and protein facilitated the transcription process,[53,54] while inhibition of the ubiquitin-mediated protein degradation inhibited the ubiquitin-dependent proteasomal degradation system and enhancing posttranslational protein.[54] During the process of xenobiotic metabolisms by CYP2E1, oxygen-free radicals were produced as by-products. Hence, induction of *Cyp2e1* might result in hepatic oxidative injury via production of oxygen-free radicals.[46,48] In this study, the expression levels of *Cyp2e1* mRNA and its activity were not significantly modified by either the *P. indica* extract or plumbagin, except an increase in activity by the *P. indica* extract at the lowest dose of 20 mg/kg/day. These evidences might be explained by the induction of *Cyp2e1* at the posttranscriptional levels, without a significant increase in the gene content, as *Cyp2e1* is inducible by a variety of small molecules.[47] This, though through an unknown mechanism, is similar to the effects of St. John's Wort extract on CYP2E1 activity in human hepatocytes where lower doses showed induction while higher doses did inhibition.[55] On the other word, the inductive effect on CYP2E1 activity was not caused by plumbagin as plumbagin did not contribute to the same effect, but produced by other constituents in the *P. indica* extract, possibly the ones also found in St. John's wort.

CYP3A takes part as 30% of the CYP450 content in the liver and responsible for metabolism of 50% clinical drugs, e.g., glucocorticoid and antiglucocorticoid hormones, macrolides, imidazole, phenobarbital, and phenobarbital-like agents.[56-58] CYP3A has low substrate specificity, meaning to be able to bind with substrates of various sizes, shapes, and chemical properties and subsequently to metabolize a variety of substrates including drugs, chemicals, and food constituents, such as polyphenols, commonly found in fruits and vegetables.[59] These make developing a new drug necessary to evaluate the metabolism pathway via CYP3A4 to predict a risk of drug interaction. Mouse CYP3A11 and CYP3A13 were the most similar to human CYP3A4, with 72 and 75% amino acid homology, respectively.[59] In the present study, the *P. indica* extract and plumbagin significantly suppressed the expression levels of *Cyp3a11* and *Cyp3a13* mRNAs and proteins in a dose-dependent pattern. In addition, both of the *P. indica* extract and plumbagin dose-dependently declined the CYP3A activity via inhibition of the erythromycin *N*-demethylation. Correspondingly, plumbagin moderately inhibited CYP3A4 activity in human liver microsomes.[38] Therefore, the evidence of inhibitory effects of *P. indica* and plumbagin on *Cyp3a11* and *Cyp3a13* should be concern since it may cause potential herb–drug interactions due to a wide variety of CYP3A substrates. These possible potential interaction outcomes due to CYP3A inhibition are such as ventricular arrhythmia associated with QT prolongation by astemizole or cisapride, symptomatic hypotension by dihydropyridine calcium antagonists or sildenafil, and excessive sedation of benzodiazepine or nonbenzodiazepine hypnosedatives.[60] Thus, administration of the *P. indica* extracts or plumbagin concomitantly with these drugs should be of high concerns.

CONCLUSIONS

CYP450s-modulatory capacities of the *P. indica* extract and plumbagin were herewith reported. In brief, *Cyp1a2* and *Cyp2e1* were induced while *Cyp2d9* and *Cyp3a11*/13 were suppressed with *Cyp2c29* being remained unchanged. Since *P. indica* is listed in the Thai national herbal formula and probably consumed with modern medicine, a potential risk of drug–herb interaction might occur. Therefore, a practitioner should be cautious of use of either *P. indica* or plumbagin-containing supplement, especially at high quantity or for a long period, for hepatotoxicity and drug interaction.

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Conflicts of interest

There are no conflicts of interest.

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