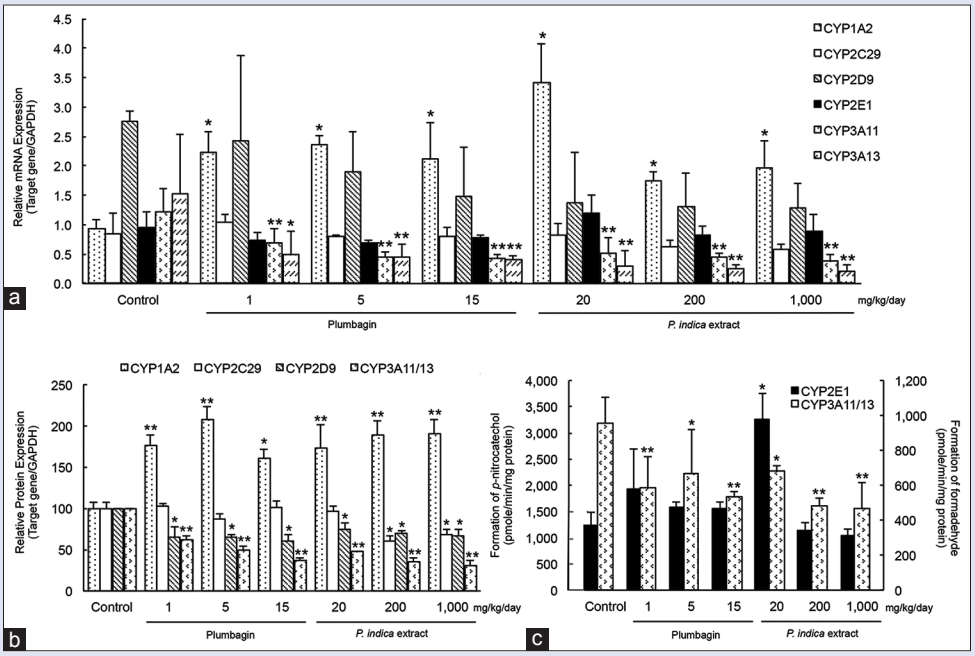

Figure 2: Effects of plumbagin and the *Plumbago indica* extract on the cytochrome P450 profiles. (a) Relative mRNA expression of cytochrome P450/glyceraldehyde 3-phosphate dehydrogenase, (b) relative protein expression of cytochrome P450/glyceraldehyde 3-phosphate dehydrogenase, and (c) formation of *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.

Acknowledgements

Nadta Sukkasem sincerely thanks the Research Group for Pharmaceutical Activities of Natural Products using Pharmaceutical Biotechnology, Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand, for scholarship and research grant and facilities. Dr. Glenn Borlace is acknowledged for critical English editing.

Financial support and sponsorship

The study was supported by the Research Group for Pharmaceutical Activities of Natural Products using Pharmaceutical Biotechnology, Khon Kaen University, Khon Kaen 40002 Thailand.

Conflicts of interest

There are no conflicts of interest.

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