



Research article

Modulatory effects of Benjakul extract on rat hepatic cytochrome P450 enzymes

Suchittra Samuhasaneeto^{a,*}, Gorawit Yusakul^b^a School of Medicine, Walailak University, Nakhon Si Thammarat, 80160, Thailand^b School of Pharmacy, Walailak University, Nakhon Si Thammarat, 80160, Thailand

ARTICLE INFO

Keywords:

Bejakul
Cytochrome P450
CYP2C11
CYP3A1
Herb-drug interaction

ABSTRACT

Benjakul, a traditional Thai formulation, has been used as a carminative and adaptogenic drug. It consists of five plants, *Piper chaba* Hunter, *Piper sarmentosum* Roxb., *Piper interruptum* Opiz., *Plumbago indica* Linn., and *Zingiber officinale* Roscoe, in equal ratios. Some individual herbs present in Benjakul were reported to modulate cytochrome P450 (CYP) enzymes. This study aimed to investigate the effects of Benjakul extract on the activities and mRNA expression levels of hepatic CYP2C11 and CYP3A1 in rats. Adult male rats were orally administered 200, 400, or 600 mg/kg BW Benjakul extract for 28 days. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN) and creatinine levels were assayed. CYP2C11 and CYP3A1 activities were analyzed using cytochrome P450 assay kits. The mRNA expression of *CYP2C11* and *CYP3A1* was measured using a quantitative real-time PCR assay. Benjakul treatment significantly increased the serum ALT and BUN levels. At doses of 200, 400, and 600 mg/kg BW, Benjakul treatment increased hepatic CYP3A1 activity and *CYP3A1* mRNA expression. *CYP2C11* mRNA expression was unchanged by treatment with Benjakul extract; however, treatment with the high and middle doses of Benjakul extract increased CYP2C11 activity. Treatment with Benjakul extract induced CYP2C11 and CYP3A1 activity in rats. Concurrent use of Benjakul with conventional drugs should be considered to potentially induce herb-drug interactions.

1. Introduction

The use of herbal medicines as complimentary or alternative medicines has become increasingly common in many countries worldwide due to scientific support for their therapeutic effectiveness and fewer harmful side effects than conventional therapies [1]. The increasing use of herbal medicine with prescription medicine has raised concerns about herb-drug interactions [2, 3, 4]. Cytochrome P450 (CYP) enzymes are members of a superfamily of hemoproteins and are widely located on the endoplasmic reticulum in liver cells [5]. They play a key role in the oxidative metabolism (phase I) of clinical drugs, other xenobiotics and endogenous substances. More than 50 CYP enzymes have been identified in humans. In the human liver, the most important CYPs for drug metabolism are members of the CYP2C and CYP3A subfamilies [6]. CYP2C9 and CYP3A4 play dominant roles in the metabolism of drugs and other xenobiotics [6]. The combination of herbal medicines with conventional drugs may affect drug metabolism because herbal medicines have the potential to induce or inhibit CYP enzymes [7].

Benjakul is a traditional Thai formulation that consists of equal amounts of five plants: the fruits of *Piper chaba* Hunter, the roots of *Piper sarmentosum* Roxb., the stems of *Piper interruptum* Opiz., the roots of *Plumbago indica* Linn. and the rhizomes of *Zingiber officinale* Roscoe. Benjakul is indicated as an herbal medicine and a carminative and adaptogenic drug in the Thailand National List of Essential Medicines [8]. In Southern Thai folk medicine, Benjakul is used prior to chemotherapy to balance patients' symptoms. Phytochemical analysis of the Benjakul extract revealed various chemical compounds, such as myristicin, plumbagin, methyl piperate, 6-shogaol, 6-gingerol and piperine [9]. Pharmacological studies have shown that Benjakul and its isolated compounds possess a wide range of pharmacological activities, including anticancer, cytotoxic, anti-inflammatory, antiallergic and antidiabetic activities [9, 10, 11, 12, 13]. Recently, the Benjakul extract showed clinical efficacy and safety in relieving symptoms in the knees of patients with primary osteoarthritis [14]. Currently, limited data are available on the interaction of Benjakul with CYP enzymes. Only the active ingredients of individual plants have been reported to modulate CYP. In vitro microsomal incubation experiments revealed that piperine inhibits

* Corresponding author.

E-mail address: ssuchitt@wu.ac.th (S. Samuhasaneeto).<https://doi.org/10.1016/j.heliyon.2021.e08498>

Received 21 June 2021; Received in revised form 4 October 2021; Accepted 25 November 2021

2405-8440/© 2021 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

CYP3A4, CYP2C9 and CYP1A2 [15]. 6-Gingerol, an active ingredient of *Z. officinale*, potently inhibits CYP2C9 activity and moderately inhibits CYP2C19 and CYP3A4 activity [16]. Therefore, assessments of the ability of Benjakul to inhibit or induce CYP2C9 and CYP3A4 activities are important. Rat CYP2C11 and CYP3A1 are homologous to human CYP2C9 and CYP3A4 [17]. Therefore, the results obtained in rats may have clinical applications. The purpose of this study was to investigate the effects of Benjakul extract on the activities and mRNA expression levels of hepatic CYP2C11 and CYP3A1 in a rat model.

2. Materials and methods

2.1. Chemicals

Benjakul powder was purchased from V.P. Pharmacy Ltd. (Bangkok, Thailand). 6-Gingerol was acquired from Glentham Life Sciences Ltd. (Corsham, UK). Piperine was obtained from Acros Organics (Belgium). Microsome isolation kits and CYP3A4 activity assay kits were purchased from Abcam (MA, USA). A CYP2C9 activity assay kit was purchased from Biovision (CA, USA). GENEzol™ reagent was acquired from Geneaid Biotech Ltd. (Taiwan). An iScript cDNA synthesis kit was obtained from Bio-Rad (CA, USA). The 5X HOT FIREPol® EvaGreen® qPCR Mix Plus (ROX) was purchased from Solis BioDyne (Estonia).

2.2. Preparation of the Benjakul extract

Two kilograms of Benjakul powder were macerated three times for three days each with 20 L of 95% ethanol at room temperature. The extract was filtered through No. 1 Whatman filter paper and then concentrated with a vacuum rotary evaporator to remove the ethanol. The extract yield was 5.9% of the Benjakul dry power. The ethanolic extract of the Benjakul formulation was stored at -20 °C until use in experiments.

2.3. High-performance liquid chromatography (HPLC) analysis of Benjakul extract

The Benjakul chemical fingerprint was obtained using a Thermo Scientific Dionex Ultimate 3000 HPLC system (Thermo Scientific, MA, USA). The instrument was composed of a tertiary pump (LPG-4300SD), an autosampler (WPS-3000SL), a column compartment (TCC-3000SL), and a variable wavelength detector (VWD-3100). Ten milligrams of Benjakul extract were dissolved in 1 mL of methanol and filtered through a 0.22 µm membrane filter before injection. The chemicals in the Benjakul extract were separated on a reverse-phase analytical column (VertiSep™ USP C18 HPLC column, 4.6 × 250 mm, 5 µm particle size; Vertical Chromatography Co., Ltd., Nonthaburi, Thailand). The mobile phases consisted of acetonitrile (solvent A) and 1% (v/v) acetic acid solution (solvent B). The gradient flow was 0 min, 20% (v/v) solvent A in solvent B. Then, the proportion of solvents was increased to 75% over 37.5 min. When the target compounds were eluted, the mobile phase was re-equilibrated with the initial condition (20%, v/v solvent A). The flow rate and injection volume were set to 1.0 mL/min and 10 µL, respectively. Detection was performed at UV-Vis wavelengths of 282 nm for 6-gingerol and 341 nm for piperine. Two markers of Benjakul extract were identified by comparing the retention time with each of the standards. The absorbance spectrum profile was used to confirm the peak.

The sensitivity of the HPLC analysis was expressed as the limit of detection (LOD) and limit of quantification (LOQ). The precision of the analysis was determined by performing triplicate injections of concentrations of the standard solution. Then, the accuracy of the method was investigated by conducting recovery experiments. The standard was spiked into the Benjakul extract at a final concentration of 20–100 µg/mL. The total concentration of both compounds was determined, and the recovery was calculated using the following Eq. (1):

$$\text{Recovery (\%)} = (\text{Concentration in SS} - \text{Concentration in NS}) \times 100 / \text{TC spiked} \quad (1)$$

The total concentrations of the compounds in spiked (SS) and non-spiked samples (NS) were analyzed. The recovery indicates the closeness of the determined concentration to the theoretical spiked concentration (TC).

2.4. Animals

Adult male Wistar rats weighing 170–200 g were purchased from Nomura Siam International Co., Ltd. (Bangkok, Thailand). The rats were housed in a room with controlled temperature (23 ± 1 °C) and humidity (50–70%) on a 12-hour light/dark cycle, and the rats were fed a rodent chow diet and provided water ad libitum. The rats were acclimated for 1 week before use in an experiment, and the experimental protocol was approved by the Animal Ethics Committee of Walailak University.

2.5. Experimental design

Benjakul extract was dissolved in a mixed solution composed of 7% Tween 80 and 3% ethanol. The doses of Benjakul extract used in this experiment were based on the doses used in humans, as described in the Thailand National List of Essential Medicines [8]. Benjakul is administered in doses of 800–1,000 mg three times a day (2,400–3,000 mg/day or 40–50 mg/kg BW/day). In the present study, the dose of 200 mg/kg BW was calculated to be the human equivalent dose of 32 mg/kg BW or approximately 1,920 mg/60 kg BW [18]. Then, the dose was doubled to calculate the other doses (400 and 600 mg/kg BW). The rats were randomly divided into four groups of six each. The treated groups were orally administered Benjakul extract daily at doses of 200, 400, or 600 mg/kg BW for 28 days. The control group was administered a daily oral dose (1 mL) of a mixed solution composed of 7% Tween 80 and 3% ethanol for 28 days. At the end of the experiment, the rats were anesthetized using Zoletil™ (25 mg/kg BW), and blood samples were collected from the heart by cardiac puncture for biochemical studies. The livers were removed immediately and rinsed with ice-cold 0.9% NaCl. The liver samples were stored at 80 °C until use in hepatic CYP2C11 and CYP3A1 analyses.

2.6. Analysis of serum biochemical parameters

Blood samples were centrifuged at 2,000 × g for 15 min, and sera were collected to test liver and kidney function. The serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN) and creatinine levels were measured using kinetic methods. All measurements were performed using an ABX Pentra 400 automated biochemistry analyzer (Horiba Medical, France).

2.7. CYP2C11 and CYP3A1 activity assays

2.7.1. Preparation of rat liver microsomes

Liver microsomes were isolated using a microsome isolation kit (Abcam, MA, USA). Briefly, 500 mg of liver were homogenized with 1 mL of cold homogenization buffer containing 2 µL/mL protease inhibitors and centrifuged at 10,000 × g for 15 min. The supernatant was collected and centrifuged at 100,000 × g for 20 min at 4 °C. The resulting microsomal pellet was washed gently with homogenization buffer, and the excess buffer was discarded. Then, the microsomal pellet was resuspended in ice-cold storage buffer (250 µL). The protein concentration was determined using the Lowry method [19]. The suspended microsomes were aliquoted and stored at -80 °C for future use.

2.7.2. Analysis of CYP2C11 and CYP3A1 activities

The activities of CYP2C11 and CYP3A1 were measured using cytochrome P450 assay kits (Biovision, CA, USA and Abcam, MA, USA for

CYP2C11 and CYP3A1, respectively). The assays were performed in black 96-well microplates. Each experiment was performed in duplicate. Twenty micrograms of microsomal protein were mixed with 2 μ L of the NADPH-generating system, and the volume was adjusted to 50 μ L/reaction with assay buffer. Twenty microliters of assay buffer and inhibitor were added to the samples and inhibition controls for each sample, respectively. After an incubation at 37 °C (30 and 15 min for CYP2C11 and CYP3A1, respectively), the enzymatic reaction was initiated by adding a mixture of β -NADP⁺ and the substrate. Then, the fluorescence of the samples was immediately measured (CYP2C11; Ex/Em = 415/502 nm, CYP3A1; Ex/Em = 535/587 nm) in kinetic mode for 30 min at 37 °C. 7-HFC and resorufin were used as the standards for the calibration curves of CYP2C11 and CYP3A1, respectively. The activities of CYP2C11 and CYP3A1 in the sample were calculated from Eq. (2):

$$\text{CYP2C11 or CYP3A1 activity} = B/(\Delta TXP) \quad (2)$$

where:

B = the amount of substrate metabolized to 7-HFC (resorufin) by CYP2C11 (CYP3A1) in a sample well based on the standard (pmol);
 ΔT = linear phase reaction time T2 – T1 (min); and
 P = amount of protein in the well (mg).

2.8. Measurement of CYP2C11 and CYP3A1 mRNA expression

2.8.1. Total RNA isolation and cDNA synthesis

Total RNA was extracted from liver tissues using GENEzol™ reagent (Geneaid Biotech Ltd, Taiwan) according to the manufacturer's instructions. The RNA pellet was dissolved in RNase-free water, and the total RNA concentration was determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). One microgram of total RNA was converted to cDNAs using an iScript cDNA synthesis kit (Bio-Rad, CA, USA). The cDNA templates were used for quantitative real-time PCR (qRT-PCR).

2.8.2. Quantitative real-time polymerase chain reaction (qRT-PCR)

qRT-PCR was performed in triplicate using the StepOnePlus™ Real-Time PCR System (Applied Biosystems, USA). The sequences of the forward and reverse primers used in this experiment are shown in Table 1. Reactions were performed in a final volume of 20 μ L that contained 10 ng of cDNAs, 5X HOT FIREPol® EvaGreen® qPCR Mix Plus (ROX) (Solis BioDyne, Estonia), 250 nM forward primer, 250 nM reverse primer and PCR-grade H₂O. The qRT-PCR conditions were as follows: initial denaturation at 95 °C for 12 min, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 56 °C for 20 s, and extension at 72 °C for 20 s. β -Actin was used as the endogenous control to normalize the target gene expression levels. The data were analyzed using the 2^{- $\Delta\Delta C_t$} method.

2.9. Statistical analysis

The results are reported as the means \pm standard deviations (SD). Differences between the control and treated groups were assessed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Kruskal-Wallis tests were conducted if the data had a nonnormal

distribution or homogeneity of variance. *P* values <0.05 were considered statistically significant for all data.

3. Results

3.1. Quantification of the two marker compounds in Benjakul extract

The chromatograms of the 6-gingerol (1) and piperine (2) standards are shown in Figure 1, and the retention times were 26.161 and 28.899 min, respectively (Figure 1A,B). The peaks of 6-gingerol (1) and piperine (2) in the Benjakul extract were identified (1C and 1D). The LOD, LOQ, linearity range of the calibration curve, precision, and analytical method accuracy are shown in Table 2.

The analytical performance indicated the reliable character of HPLC for this analysis. The analysis revealed that 3.50 \pm 0.03 μ g/mg 6-gingerol and 88.7 \pm 0.48 μ g/mg piperine were present in the Benjakul extract.

3.2. Effect of Benjakul extract on serum biochemical parameters

After feeding Benjakul extract to the rats for 28 days, the Benjakul-treated groups (400 and 600 mg/kg BW) exhibited increased serum ALT levels (*P* < 0.05) compared to the control group. The serum BUN levels were significantly increased in the Benjakul-treated groups (200, 400 and 600 mg/kg BW) compared with the control group (*P* < 0.05). No significant differences in AST and creatinine levels were observed among the experimental groups (Table 3).

3.3. Effect of Benjakul extract on CYP2C11 and CYP3A1 activities

The effects of Benjakul extract on CYP2C11 and CYP3A1 activities are shown in Figure 2. Compared with the control condition, treatment with 400 and 600 mg/kg BW Benjakul increased rat hepatic CYP2C11 activity (*P* < 0.05) (Figure 2A). No significant change in CYP2C11 activity was detected after low-dose Benjakul treatment. Additionally, compared with the control condition, the administration of Benjakul extract (200, 400 and 600 mg/kg BW) increased CYP3A1 activity (*P* < 0.05) (Figure 2B).

3.4. Effect of Benjakul extract on CYP2C11 and CYP3A1 mRNA expression

The CYP2C11 and CYP3A1 mRNA expression levels in the liver determined using qRT-PCR are shown in Figure 3. Compared with the control condition, Benjakul treatments (200, 400 and 600 mg/kg BW) significantly (*P* < 0.05) upregulated CYP3A1 mRNA expression (Figure 3A). However, the hepatic CYP2C11 mRNA expression levels in the Benjakul treatment groups were not different from that of the control group (*P* < 0.05) (Figure 3B).

4. Discussion

In the present study, the administration of Benjakul extract increased CYP2C11 and CYP3A1 activities and upregulated CYP3A1 mRNA expression in the rat liver. Benjakul treatment for 28 days might lead to liver and kidney injury. In addition, piperine and 6-gingerol were identified in the Benjakul extract. The piperine content in the Benjakul extract was similar to that reported in a previous study [9]. However, we detected a low 6-gingerol content in the Benjakul extract. The difference in the 6-gingerol content in this report might be due to differences in plant cultivation, harvesting period and extraction procedure.

Concurrent use of herbal plants and conventional drugs results in potential herb-drug interactions. The most common mechanisms of herb-drug interactions involve pharmacokinetic interference, namely, the induction or inhibition of CYP enzymes [7]. The induction of CYP enzymes increases drug clearance, resulting in a decreased therapeutic effect, while inhibition leads to a decrease in drug excretion and then increases the drug plasma concentration, potentially leading to toxicity.

Table 1. Primer sequences used to analyze CYP2C11 and CYP3A1 mRNA expression [20].

Gene	Primer sequence (5'3')
CYP2C11	Forward: AAAAGCACAAATCCGAGTCT
	Reverse: GCATCTGGCTCCTGTCTTTTC
CYP3A1	Forward: TCTGTGCAGAAGCATCGAGTG
	Reverse: TGGGAGGTGCCTTATTGGG
β -Actin	Forward: GACCTCTATGCCAACACAGT
	Reverse: GGTGTA AACGCAGCTCAGTA

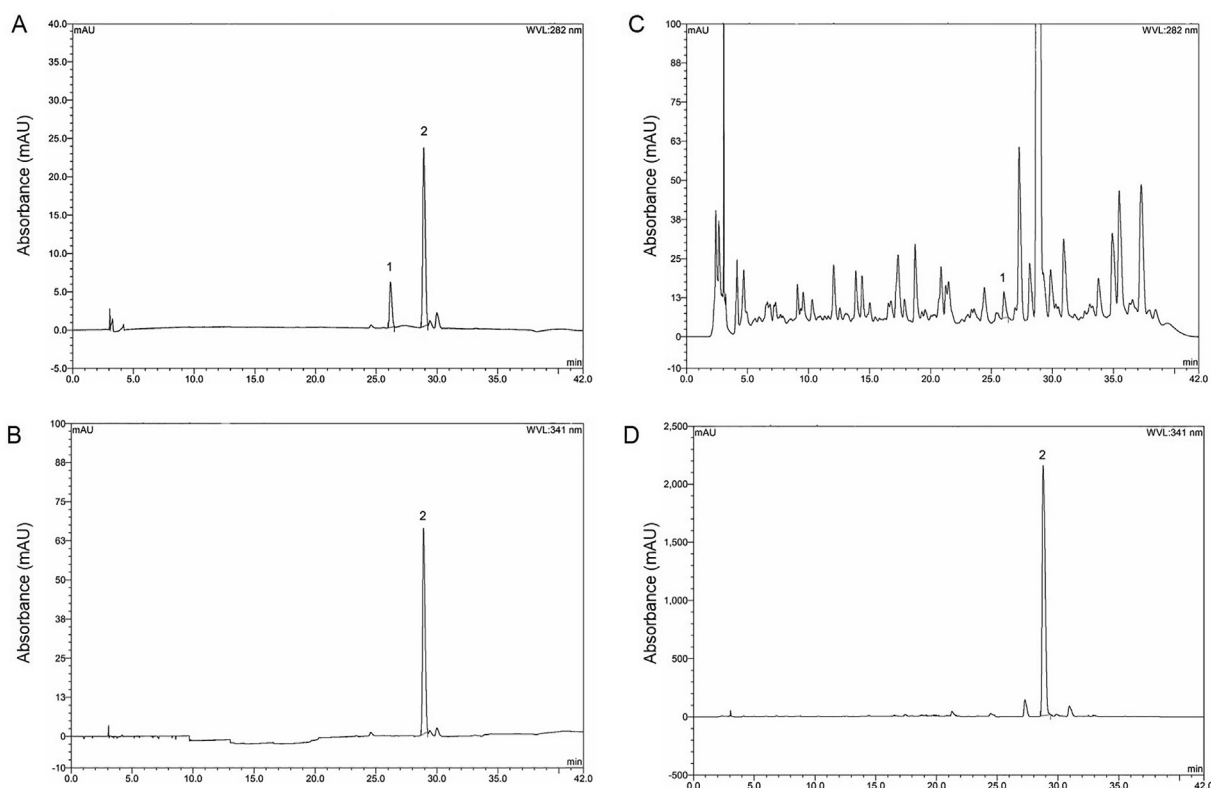


Figure 1. Chromatograms of the standards (25 $\mu\text{g/mL}$) of 6-gingerol (1), and piperine (2), which were detected at 282 nm (A) and 341 nm (B), respectively. Both compounds were identified in the sample solution of Benjakul extract (10 mg/mL), in which 6-gingerol (1) and piperine (2) were detected at 282 nm (C) and 341 nm (D), respectively.

Table 2. The analytical performance of HPLC for 6-gingerol (1) and piperine (2) determinations.

Analytical parameters	Analyte	
	6-gingerol (1)	piperine (2)
Limitation of detection (LOD)	0.204 $\mu\text{g/mL}$	0.916 $\mu\text{g/mL}$
Limitation of quantification (LOQ)	0.618 $\mu\text{g/mL}$	2.775 $\mu\text{g/mL}$
Linearity range of calibration curve (R^2)	1.56–100 $\mu\text{g/mL}$ ($R^2 = 0.9997$)	3.13–100 $\mu\text{g/mL}$ ($R^2 = 0.9993$)
Precision (% CV)	0.13–0.92%	0.12–4.40%
Accuracy (% recovery)	97.5–109%	98.7–104%

Table 3. Effect of Benjakul extract on serum biochemical parameters.

Group	ALT (U/L)	AST (U/L)	BUN (mg/dL)	Creatinine (mg/dL)
Control	20.67 \pm 2.42	83.00 \pm 5.62	14.17 \pm 1.17	0.30 \pm 0
BJK 200	20.33 \pm 2.16	78.83 \pm 6.31	19.00 \pm 2.10*	0.27 \pm 0.05
BJK 400	24.83 \pm 2.14*	73.00 \pm 7.56	19.17 \pm 1.72*	0.30 \pm 0
BJK 600	31.33 \pm 8.94*	76.00 \pm 10.18	19.83 \pm 2.86*	0.30 \pm 0

Values are presented as the means \pm SD (n = 6). * $P < 0.05$ compared with the control group. BJK 200, 200 mg/kg BW Benjakul; BJK 400, 400 mg/kg BW Benjakul; BJK 600, 600 mg/kg BW Benjakul.

The CYP3A subfamily comprises more than 40% of all CYP enzymes in humans [7]. CYP3A4 is most abundantly expressed isoform in the liver and participates in the metabolism of 50% of clinical drugs, such as macrolide antibiotics, midazolam, immune system modulators, and calcium channel blockers. Regarding the metabolism of endogenous substances, CYP3A4 metabolizes testosterone, progesterone, and

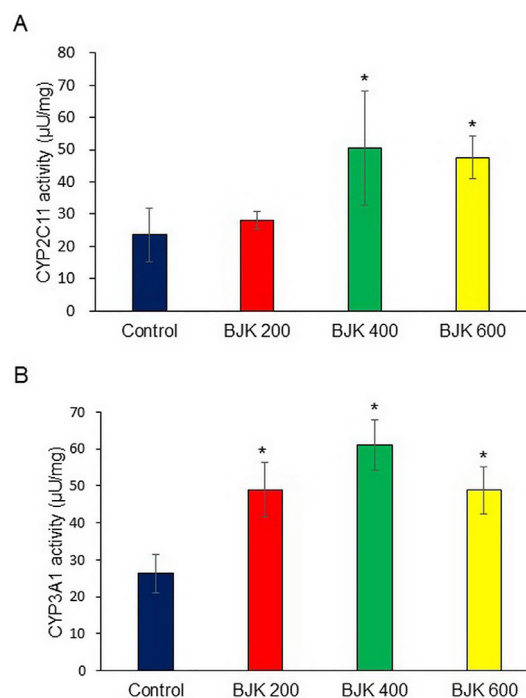


Figure 2. Effect of Benjakul extract on CYP2C11 activity (A) and CYP3A1 activity (B). Values are reported as the means \pm SD (n = 6). * $P < 0.05$ compared with the control group. BJK 200, 200 mg/kg BW Benjakul; BJK 400, 400 mg/kg BW Benjakul; BJK 600, 600 mg/kg BW Benjakul.

androstenedione [5]. Human CYP3A4 is homologous to rat CYP3A1 [17]. Thus, rodent models are commonly used to predict human CYP3A4

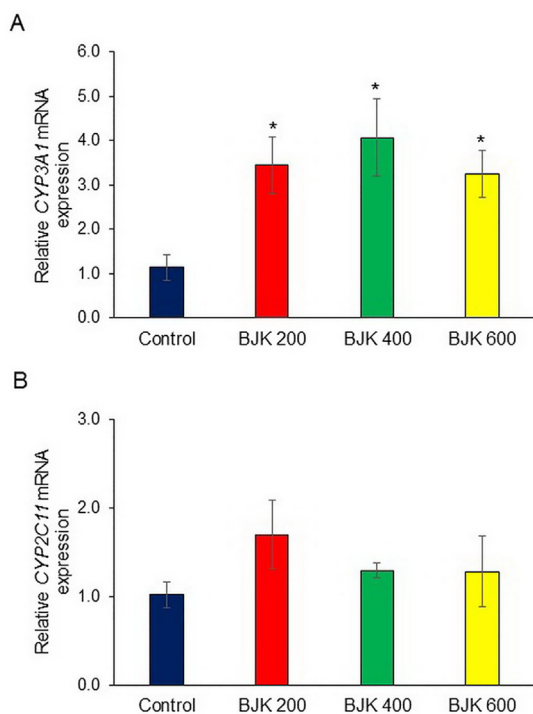


Figure 3. Relative *CYP3A1* (A) and *CYP2C11* (B) mRNA expression levels in different experimental groups of rats (n = 4). The mRNA levels of *CYP3A1* and *CYP2C11* were normalized to the β -actin level. BJK 200, 200 mg/kg BW Benjakul; BJK 400, 400 mg/kg BW Benjakul; BJK 600, 600 mg/kg BW Benjakul.

activity in vivo. No report has assessed the effect of Benjakul extract on CYP3A4. Some individual herbs present in Benjakul formulations or active ingredients were shown to inhibit CYP3A4. For example, *P. indica* and one of its active compounds (plumbagin) suppress the mRNA and protein expression of *CYP3A11* in mice [21]. *P. chaba* and *P. indica* potentially inhibit CYP3A4 activity in human liver microsomes [22]. In addition, piperine, an active compound of *P. chaba*, inhibits recombinant human CYP3A4 [15]. In HepG2 cells, 6-gingerol, an active ingredient of *Z. officinale*, moderately inhibits CYP3A4 [16]. In the present study, mRNA levels and enzyme activities were measured to evaluate both enzyme inhibition and induction during Benjakul treatment. The Benjakul extract treatment significantly increased CYP3A1 activity and *CYP3A1* mRNA expression in rats. The Benjakul formulation is composed of five herbal plants that contain several bioactive compounds. The formulation induced CYP3A1 expression and activity. *CYP3A4* expression is regulated by ligand-activated nuclear receptors such as pregnane nuclear receptor (PXR) and constitutive androstane receptor (CAR) [23]. Piperine activates human PXR to induce CYP3A4 expression in human hepatocytes. In another study, piperine increased PXR activity in human hepatoma cells [24]. In the present study, piperine was identified as a major compound in Benjakul extract. Therefore, the mechanism by which Benjakul extract mediates the upregulation of *CYP3A1* expression and activity may involve the activation of PXR. Recently, Benjakul has been used to treat patients with allergic rhinitis and lung cancer [25, 26]. Herb-drug interactions may occur if Benjakul extract is combined with drugs metabolized by CYP3A4. For example, if it is used in combination with loratadine or paclitaxel, it may decrease drug plasma concentrations to subtherapeutic levels, leading to therapeutic failure.

CYP2C9 is a member of the CYP2C subfamily that is expressed at high levels in the human liver [27]. It metabolizes approximately 15% of all drugs, such as the anticoagulant warfarin, the antidiabetic agent tolbutamide, nonsteroidal anti-inflammatory drugs, angiotensin II blockers and antiepileptics [27]. Rat CYP2C11 is functionally similar to human CYP2C9. In the present study, the *CYP2C11* mRNA expression level was not significantly modified by Benjakul extract, but Benjakul extract did

increase CYP2C11 activity at doses of 400 and 600 mg/kg BW. Some studies reported that gene expression levels are not correlated with CYP activities [28, 29]. Moreover, increased CYP2C9 activity might be regulated at the posttranscriptional level. Thus, further investigations of the mechanism underlying this effect are necessary. Our results reveal that Benjakul extract increases CYP2C11 activity. Therefore, coadministration of Benjakul extract with drugs metabolized by CYP2C9, i.e., warfarin, tolbutamide, diclofenac and phenytoin, may increase the elimination of these drugs and decrease the therapeutic effect. For instance, concomitant use of Benjakul extract and warfarin may decrease plasma concentrations and reduce the clinical effects of warfarin.

In addition to investigating the safety of Benjakul treatment in rats, serum biochemical markers of liver and kidney damage were determined. The doses of Benjakul extract administered to the animals were based on the Benjakul doses used in humans [8]. A Benjakul dose of 200 mg/kg BW can be converted into the human equivalent dose of 32 mg/kg BW or approximately 1,920 mg/60 kg BW [18]. Benjakul extract doses ranging from 300 to 1,200 mg/kg were used in animal studies [11, 13]. High-dose and moderate-dose Benjakul extract treatments increased serum ALT levels, indicating hepatocellular damage. Likewise, BUN levels were increased in Benjakul-treated rats, which indicates kidney injury. These findings differ from those of a previous toxicity test. The administration of Benjakul extract for 6 months had no effect on the tested animals or their blood chemistry [30]. The doses administered in our study (400 and 600 mg/kg BW) may be sufficiently high to induce hepatotoxicity and nephrotoxicity in rats. Therefore, the use of high doses and long-term treatment with Benjakul should be avoided.

Our study has a few limitations. First, as species-specific differences exist for drug-metabolizing enzymes, our results may not accurately reflect the potential to induce CYP activity in humans. In addition, an initial Benjakul extract dose of 200 mg/kg BW induced CYP activity in rats. We were unable to clearly determine whether doses lower than 200 mg/kg will affect CYPs in rats. Thus, studies using a range of doses in animal models should be performed. Last, several substances are present in Benjakul extract; however, information about active substances involved in CYP induction is limited. Further studies of Benjakul extract should focus on the substances in Benjakul extract involved in CYP induction and PXR activation. Furthermore, human studies are needed to confirm the interactions of Benjakul extract with CYP isoforms in humans.

In conclusion, the data in this study indicate that Benjakul extract activates CYP2C11 and CYP3A1 in rats. Coadministration of Benjakul with conventional drugs should be considered to potentially induce herb-drug interactions.

Declarations

Author contribution statement

Suchitra Samuhasaneeto: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Gorawit Yusakul: Analyzed and interpreted the data; Wrote the paper.

Funding statement

This work was supported by a grant from Walailak University, grant number WU-IRG-63-051.

Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

- [1] T. Wegener, Patterns and trends in the use of herbal products, herbal medicine and herbal medicinal products, *Int. J. Compl. Alt. Med.* 9 (2017) 317.
- [2] P.S. Fasinu, G.K. Rapp, Herbal interaction with chemotherapeutic drugs—a focus on clinically significant findings, *Front. Oncol.* 9 (1356) (2019).
- [3] T.B. Agbabiaka, N.H. Spencer, S. Khanom, C. Goodman, Prevalence of drug-herb and drug-supplement interactions in older adults: a cross-sectional survey, *Br. J. Gen. Pract.* 68 (2018) e711–e717.
- [4] A. Ramos-Esquivel, Á. Viquez-Jaikel, C. Fernández, Potential drug-drug and herb-drug interactions in patients with cancer: a prospective study of medication surveillance, *J. Oncol. Pract.* 13 (2017) e613–e622.
- [5] P.B. Danielson, The cytochrome P450 superfamily: biochemistry, evolution and drug metabolism in humans, *Curr. Drug Metab.* 3 (2002) 561–597.
- [6] S.E. Leucuta, L. Vlase, Pharmacokinetics and metabolic drug interactions, *Curr. Clin. Pharmacol.* 1 (2006) 5–20.
- [7] B. Rosenkranz, P. Fasinu, P. Bouic, An overview of the evidence and mechanisms of herb–drug interactions, *Front. Pharmacol.* 3 (69) (2012).
- [8] Drug and medical supply information [internet], Minist. Publ. Health (2021) [cited 2021 Apr 13]. Available from: <http://dmsic.moph.go.th/index/dataservice/97/0>.
- [9] R. Rattarom, I. Sakpakdeejaroen, P. Hansakul, A. Itharat, Cytotoxic activity against small cell lung cancer cell line and chromatographic fingerprinting of six isolated compounds from the ethanolic extract of Benjakul, *J. Med. Assoc. Thai.* 97 (Suppl 8) (2014) S70–S75.
- [10] A. Itharat, R. Rattarom, P. Hansakul, I. Sakpakdeejaroen, B. Ooraikul, N. Davies, The effects of Benjakul extract and its isolated compounds on cell cycle arrest and apoptosis in human non-small cell lung cancer cell line NCI-H226, *Res. Pharm. Sci.* 16 (2021) 129–140.
- [11] P. Kuropakornpong, A. Itharat, S. Panthong, S. Sireeratawong, B. Ooraikul, In vitro and in vivo anti-inflammatory activities of benjakul: a potential medicinal product from Thai traditional medicine, *Evid Based Compl. Alt. Med. : eCAM* 2020 (2020) 9760948.
- [12] S. Makchuchit, R. Rattarom, A. Itharat, The anti-allergic and anti-inflammatory effects of Benjakul extract (a Thai traditional medicine), its constituent plants and its some pure constituents using in vitro experiments, *Biomed. Pharmacother.* 89 (2017) 1018–1026.
- [13] K. Vongthoung, A. Kamchansuppasin, P. Temrangsee, N. Munkong, N. Kaendee, N. Lerdvuthisophon, Effects of Benjakul water extract on pancreas in high-fat fed rats, *TMJ* 16 (2016) 161–175.
- [14] P. Rachawat, P. Pinsornsak, P. Kanokkangsadal, A. Itharat, Clinical efficacy and safety of benjakul remedy extract for treating primary osteoarthritis of knee compared with diclofenac: double blind, randomized controlled trial, *Evid Based Compl. Alt. Med. : eCAM* 2017 (2017) 9593580.
- [15] S. Shamsi, H. Tran, R.S.J. Tan, Z.J. Tan, L.Y. Lim, Curcumin, piperine, and capsaicin: a comparative study of spice-mediated inhibition of human cytochrome P450 isozyme activities, *Drug Metab. Dispos.* 45 (2017) 49–55.
- [16] M. Li, P-z Chen, Q-x Yue, J-q Li, R-a Chu, W. Zhang, et al., Pungent ginger components modulates human cytochrome P450 enzymes in vitro, *Acta Pharmacol. Sin.* 34 (2013) 1237–1242.
- [17] M. Martignoni, G.M. Groothuis, R. de Kanter, Species differences between mouse, rat, dog, monkey and human CYP-mediated drug metabolism, inhibition and induction, *Expet Opin. Drug Metabol. Toxicol.* 2 (2006) 875–894.
- [18] A.B. Nair, S. Jacob, A simple practice guide for dose conversion between animals and human, *J. Basic Clin. Pharm.* 7 (2016) 27–31.
- [19] O.H. Lowry, N.J. Rosebrough, A.L. Farr, Protein measurement with the folin phenol reagent, *J. Biol. Chem.* 193 (1951) 265–275.
- [20] Y. Huang, S.L. Zheng, H.Y. Zhu, Z.S. Xu, R.A. Xu, Effects of aescin on cytochrome P450 enzymes in rats, *J. Ethnopharmacol.* 151 (2014) 583–590.
- [21] N. Sukkasem, W. Chatuphonprasert, K. Jarukamjorn, Altered cytochrome P450 profiles by *Plumbago indica* Linn. and plumbagin after oral administration in mice, *Phcog. Mag.* 14 (2018) 507–512.
- [22] W. Sumsakul, W. Mahavorasirikul, K. Na-Bangchang, Inhibitory activities of Thai medicinal plants with promising activities against malaria and cholangiocarcinoma on human cytochrome P450, *Phytother. Res.* 29 (2015) 1926–1933.
- [23] P. Manikandan, S. Nagini, Cytochrome P450 structure, function and clinical significance: a review, *Curr. Drug Target* 19 (2018) 38–54.
- [24] F. Qiang, K.W. Kang, H.K. Han, Repeated dosing of piperine induced gene expression of P-glycoprotein via stimulated pregnane-X-receptor activity and altered pharmacokinetics of diltiazem in rats, *Biopharm. Drug Dispos.* 33 (2012) 446–454.
- [25] K. Hougiam, W. Chanvimalueng, A. Itharat, N. Mukkasombut, P. Chaityawatthanananth, Quality of life of allergic rhinitis patients treated with ethanolic benjakul remedy extract compared with loratadine, *TMJ* 19 (2019) 36–45.
- [26] P. Chantor, H. Rattanabanjerdkul, A. Itharat, Case studies of Benjakul recipes in treating stage 4 non-small cell lung cancer, *TMJ* 17 (2017) 172–181.
- [27] A.K. Daly, A.E. Rettie, D.M. Fowler, J.O. Miners, Pharmacogenomics of CYP2C9: functional and clinical considerations, *J. Personalized Med.* 8 (1) (2017).
- [28] T. Yan, L. Lu, C. Xie, J. Chen, X. Peng, L. Zhu, et al., Severely impaired and dysregulated cytochrome P450 expression and activities in hepatocellular carcinoma: implications for personalized treatment in patients, *Mol. Cancer Therapeut.* 14 (2015) 2874–2886.
- [29] V. Clermont, A. Grangeon, A. Barama, J. Turgeon, M. Lallier, J. Malaise, et al., Activity and mRNA expression levels of selected cytochromes P450 in various sections of the human small intestine, *Br. J. Clin. Pharmacol.* 85 (2019) 1367–1377.
- [30] A. Itharat, S. Sireeratawong, S. Vannasiri, J. Charoenphandhu, K. Siyakun, P. Tappayutpijarn, Development of Benjakul Extract as Health Products for Cancer Patients, Thammasat University, 2010.