Tropical Journal of Pharmaceutical Research December 2021; 20 (12): 2519-2525
**ISSN:** 1596-5996 (print); 1596-9827 (electronic)


Available online at http://www.tjpr.org
**http://dx.doi.org/10.4314/tjpr.v20i12.9**

**Original Research Article**

# Potential of *Anethum graveolens* L. to up-regulate the expression of phase I and II metabolizing genes in HepG2 cells

**Wachirawit Udomsak[1], Waranya Chatuphonprasert[1,2], Wipawee Tukum-mee[3], Jintanaporn Wattanathorn[3], Kanokwan Jarukamjorn[1]***

*[1]Research Group for Pharmaceutical Activities of Natural Products using Pharmaceutical Biotechnology (PANPB), Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen 40002, [2]Faculty of Medicine, Mahasarakham University, Mahasarakham 44000, [3]Research Institute for Human High Performance and Health Promotion, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand*

**For correspondence: **Email:** kanok_ja@kku.ac.th; **Tel/Fax:** +66-43-202379*

*Sent for review: 8 September 2021* *Revised accepted: 29 November 2021*

## Abstract

***Purpose:*** *To investigate the effects of the herbal medicine Anethum graveolens L. (AG) on the expressions of phase I and II metabolizing genes in HepG2 cells in order to gain insight into the metabolism of AG.*
***Methods:*** *HepG2 cells (5 x $10^5$ cells/well) were treated with either 10 µM ketoconazole, 20 µM rifampicin, or AG extract (60 - 480 µg/mL) for 72 h. Cell viability and reactive oxygen species (ROS) production were assessed using resazurin and 2',7'-dichlorofluorescein diacetate assays, respectively. The mRNA expression of phase I (CYP1A2, CYP2C19, CYP2D6, and CYP3A4) and phase II metabolizing enzymes (UGT1A6 and NAT1) were determined using reverse transcription-real-time polymerase chain reaction (qRT-PCR).*
***Results:*** *ROS production was not affected by the various treatments. The highest concentration of AG (480 µg/mL) reduced cell viability to 63.33 %. The expressions of CYP1A2, CYP2C19, CYP2D6, and NAT1 mRNA were significantly elevated after 72-h AG treatment.*
***Conclusion:*** *The use of AG as an alternative medicine, particularly at high concentrations and/or after prolonged use, poses a risk for herb-drug interactions due to the up-regulation of CYP1A2, CYP2C19, CYP2D6, and NAT1 expression by AG in HepG2 cells.*

***Keywords:*** *Dill, Reactive oxygen species, Metabolizing enzymes, Drug interaction, Hepatocellular carcinoma cell*



**Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts**

## INTRODUCTION

*Anethum graveolens* L. (AG; dill or Phak Chee Lao in Thai; Figure 1) is an annual plant of the family Apiaceae (Umbelliferae) that is widely cultivated in Europe, North America, and Asia. AG is extensively used as a condiment, as an ingredient in vinegars and pastries, and as an herbal medicine in treating and preventing various ailments due to its anti-hyperlipidemic,

anti-glycemic, anti-microbial, anti-inflammatory, analgesic, anti-secretory, anti-spasmodic, and anti-oxidant properties [1]. Extracts of AG were shown to reduce serum cholesterol levels and prevent insulin sensitization in clinical trials [2], and have shown hepatoprotective and antioxidant activities in hypercholesterolemic N-Mary rats [3]. Furthermore, essential oils from AG have exhibited *in vitro* antibacterial and anti-fungal activities [4]. The metabolism of AG has not been extensively studied, but two reports noted that carvone, a major essential oil of AG, affects the regulatory pathways of phase I and phase II metabolizing enzymes, which could potentially cause herb-drug interactions (HDIs) [5].


**Figure 1:** Morphology of *Anethum graveolens* L. (a), roots (b), and leaves (c)

Both phase I and phase II metabolism play critical roles protecting the body from endogenous and exogenous compounds. Cytochrome P450 (CYP450) is a chief super-family of metabolic enzymes that is mainly responsible for phase I bioactivation and detoxification of exogenous compounds. CYP450 enzymes act by catalyzing the oxidation, reduction, or hydrolysis of exogenous compounds and over 90% of drugs and xenobiotics are metabolized by five isoforms: *CYP1A2*, *CYP2C9*, *CYP2C19*, *CYP2D6*, and *CYP3A4/5* [6]. Transferases are the major enzymes in phase II metabolism. Transferases such as UDP-glucuronosyltransferases (UGTs) and *N*-acetyltransferases (NATs) facilitate the conjugation of metabolites from phase I or their parent compounds [7].

The human hepatocellular carcinoma cell line (HepG2) is commonly employed as a surrogate for human primary hepatocytes due to the limitations of working with primary cells (unpredictable availability, limited life span, high cost of operation, inter-individual variability, and complicated isolation and culture procedures) [8]. HepG2 cells are used because almost of their genes possess low basal expression and inducibility.

## EXPERIMENTAL

### Chemicals

**Dulbecco's** modified Eagle medium (DMEM) supplemented with 1 g/L D-glucose, 2.5 mM L-glutamine, 110 mg/mL sodium pyruvate, and 5 % (v/v) fetal bovine serum (FBS) was obtained from Gibco® (Life Technologies™, MA, USA). Rifampicin, ketoconazole, resazurin, and 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Forward and reverse primers for CYP1A2, CYP2C19, CYP2D6, CYP3A4, UGT1A6, NAT1, and GAPDH genes were synthesized by Bio Basic, Inc. (Markham, Ontario, Canada). All other laboratory chemicals were of the highest purity from commercial suppliers.

### Sample preparation

The AG extract (Petty patent No. 16714, Department of Intellectual Property of Thailand) was provided by the Research Institute for Human High Performance and Health Promotion, Khon Kaen University, Thailand. Briefly, aerial parts of AG were dried, powdered, and macerated for 72 h in 95 % ethanol before evaporation and freeze-drying to obtain the AG extract

### Cell culture

The HepG2 cells (ATCC® HB-8065, Manassas, USA) were seeded in a 24-well plate at density of $5 \times 10^5$ cells/well at 37 ºC, 5 % $CO_2$ and 95 % relative humidity for 48 h. Then, the cells were treated with 0.2% dimethylsulfoxide (DMSO; control), 20 μM rifampicin (a typical inducer), 10 μM ketoconazole (a typical inhibitor), or the AG extract (concentrations ranged from 60 to 480 μg/mL) for 72 h. The medium was collected for assessment of cell viability and determination of levels of reactive oxygen species (ROS). The cells were collected for preparation of total RNA and quantitative determination of mRNA expression using reverse transcription and real-time polymerase chain reaction (RT-qPCR).

### Assessment of cell viability

Cell viability was assessed using the resazurin assay. A 72-h medium was mixed with 1 mM resazurin before incubation in 5 % $CO_2$ at 37 ºC for 1 h. Cell viability (%) was calculated from the increased fluorescence intensity at excitation of 530 nm and emission of 580 nm [9].

### Determination of reactive oxygen species (ROS)

The ROS level was determined by 2′,7′-dichlorofluorescein diacetate (DCFH-DA) method [10]. The medium was mixed with 0.06 µM DCFH-DA and incubated at room temperature in the dark for 40 min. DCFH-DA reacts with ROS in the medium and generates highly fluorescent dichlorofluorescein (DCF). Fluorescence intensity was measured at excitation wavelength of 484 nm and emission of 530 nm. ROS level was calculated by comparison with a standard curve of hydrogen peroxide (2.5 - 20 µM).

### Quantitative determination of mRNA expression

Total RNA was extracted from cells using guanidinium thiocyanate-phenol-chloroform method [11]. Concentration and purity were examined at 260/280 and 260/230 nm, respectively, using a NanoDrop 2000c UV-Vis Spectrophotometer (NanoDrop Technologies, Inc., Thermo Fisher Scientific, MA, USA). Integrity of total RNA was analyzed by 1.25 % agarose gel electrophoresis. Then, total RNA was converted to cDNA using ReverTraAce® (Toyobo Co. Ltd, Osaka, Japan) at 25 ºC for 10 min, 42 ºC for 60 min, and 95 ºC for 5 min and cDNA was amplified to determine expressions of phase I CYP450s (*CYP1A2, CYP2C19, CYP2D6,* and *CYP3A4*), phase II (*UGT1A6* and *NAT1*) metabolizing enzymes, and a reference gene, *GAPDH* with specific forward and reverse primers for each gene (Table 1) under conditions recommended by the supplier [12]. The mRNA level was normalized to that of *GAPDH* and expressed as relative fold expression using delta-delta $C_t$ method.

### Statistical analysis

The results are expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way ANOVA with Tukey's statistical *post hoc* test. SPSS version 26.0 (Armonk, New York, USA) was used for the statistical analysis. Statistical difference was set at $p < 0.05$.

## RESULTS

### Effect of AG on cell viability and ROS level in HepG2 cells

After cells 72 h incubation, all treatments did not affect the viability of HepG2 cells, except for the highest concentration of AG extract, which produced a decrease to 63.33 % cell viability (Figure 2 A). The ROS levels were unchanged by all treatments including typical modifiers and AG extracts (Figure 2 B). Hence, concentrations of AG extract ranging from 60 to 240 µg/mL were employed for investigation of phase I and II metabolism in HepG2.

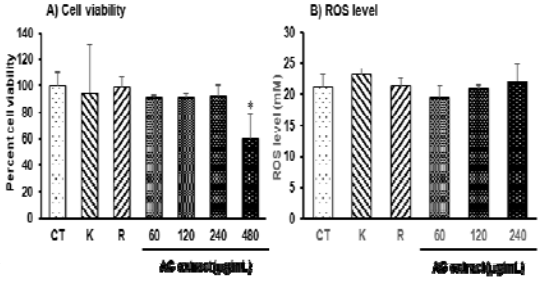


**Figure 2:** Effect of AG extract on cell viability and ROS level in HepG2 cells. CT, 0.2% DMSO; K; 10 µM ketoconazole; R; 20 µM rifampicin; AG, Anethum graveolens L. n = 4; *p < 0.05 versus CT

**Table 1:** Primer sequences for determination of human metabolizing genes

| Gene | | Forward and reverse primers (5'®3') | $T_{Annealing}$ (˚C) | Product size (bp) |
|---|---|---|---|---|
| *CYP1A2* | *F* | ACAAGGGACACAACGCTGAA | 60.0 | 160 |
| | *R* | AGGGCTTGTTAATGGCAGTG | | |
| *CYP2C19* | *F* | GGATTGTAAGCACCCCCTG | 60.0 | 174 |
| | *R* | TAAAGTCCCGAGGGTTGTTG | | |
| *CYP2D6* | *F* | AGCTTTCTGGTGACCCCATC | 61.1 | 135 |
| | *R* | GGACCCGAGTTGGAACTACC | | |
| *CYP3A4* | *F* | CTTCATCCAATGGACTGCATAAA | 55.0 | 87 |
| | *R* | TCCCAAGTATAACACTCTACACACACA | | |
| *UGT1A6* | *F* | AGCCCAGACCCTGTGTCCTA | 58.2 | 76 |
| | *R* | CCACTCGTTGGGAAAAAGTCA | | |
| *NAT1* | *F* | GAATTCAAGCCAGGAAGAAGCA | 60.0 | 151 |
| | *R* | TCCAAGTCCAATTTGTTCCTAGACT | | |
| *GAPDH* | *F* | CACCATCTTCCAGGAGCGAG | 61.1 | 72 |
| | *R* | GACTCCACGACGTACTCAGC | | |

**Effect of AG extract on mRNA expression of phase I metabolizing genes in HepG2 cells**

The effects of AG extract on the expression of CYP1A2, CYP2C19, CYP2D6, and CYP3A4 mRNA in HepG2 cells were analyzed. The 120 and 240 µg/mL concentrations of AG extract significantly up-regulated the expression of CYP1A2 (1.48 and 1.7-fold increase in expression compared to control, respectively) and CYP2C19 (2.39 and 4.15-fold increase in expression compared to control, respectively) in HepG2 cells (all $p < 0.05$; Figures 3A and 3 B). Ketoconazole extensively elevated expressions of CYP1A2 11.87-fold, while CYP1A2 expression was unchanged by rifampicin (Figure 3 A). Notably, neither ketoconazole nor rifampicin modified CYP2C19 expression (Figure 3 B). Expression of CYP2D6 was significantly induced at all concentrations of AG extract (2.00- to 2.56-fold, $p < 0.05$) and ketoconazole (5.40-fold, $p < 0.05$), while rifampicin did not affect CYP2D6 expression (Figure 3 C). Expression of CYP3A4 was significantly up-regulated by both ketoconazole (increased 6.60-fold, $p < 0.05$) and rifampicin (increased 2.96-fold, $p < 0.05$), but not by AG extract (Figure 3 D). These observations suggest that AG extract meaningfully induced the mRNA expression of CYP1A2, CYP2D6, and CYP2C19

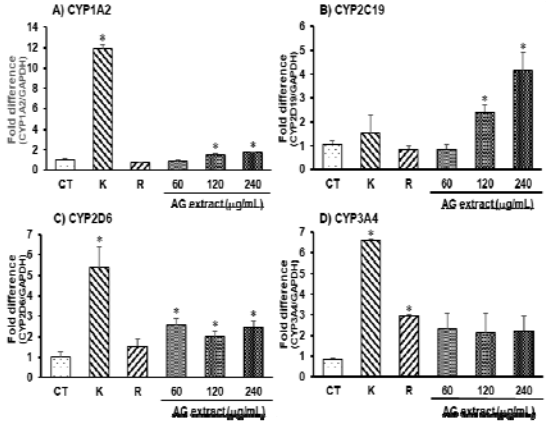


**Figure 3:** Effect of AG extract on the mRNA expressions of phase I metabolizing genes. CT, 0.2% DMSO; K; 10 µM ketoconazole; R, 20 µM rifampicin; AG, *Anethum graveolens* L. *n* = 4; **p* < 0.*05 vs* CT

**Effect of AG extract on mRNA expressions of phase II metabolizing genes in HepG2 cells**

Conjugation of parent compound(s) and/or phase I metabolite(s) are/is predominantly catalyzed by phase II metabolism [7]. Expression of *UGT1A6* was significantly induced by ketoconazole (3.77-fold, $p < 0.05$), but not by rifampicin and AG extract (Figure 4 A). Likewise, expression of *NAT1* was significantly up-regulated by ketoconazole (4.65-fold, $p < 0.05$) (Figure 4 B). Interestingly, the highest concentration of AG extract (240 µg/mL) induced expression of *NAT1*, increasing the mRNA level by 2.32-fold compared to the control ($p < 0.05$).

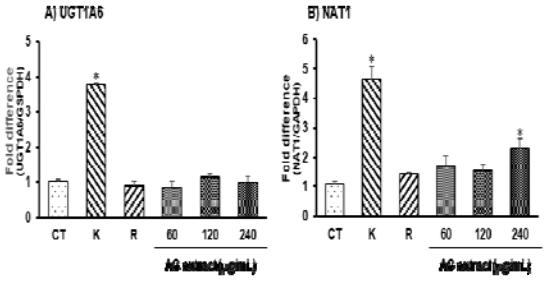


**Figure 4:** Effects of AG extract on the mRNA expressions of phase II metabolizing genes. CT, 0.2% DMSO; K, 10 µM ketoconazole; R; 20 µM rifampicin; AG; Anethum graveolens L. n = 4; *p < 0.05 versus CT.

## DISCUSSION

AG is used as an alternative medicine due to several pharmacological properties, but the safety and metabolism of AG extracts have not been determined. AG extract at the highest tested concentration (480 µg/mL) exhibited cytotoxicity in HepG2 cells after 72-hour treatment, while concentrations ranging from 60 to 240 µg/mL did not impair cell viability or increase ROS levels. Nevertheless, AG may influence the effectiveness of clinical drugs by up-regulating important genes in phase I (CYP1A2, CYP2D6, and CYP2C19) and phase II (NAT1) metabolism, which then poses a risk for the development of AG-clinical drug interactions.

As AG extract at high concentrations (120 and 240 µg/mL) induced expression of CYP1A2 and CYP2C19 mRNA polypharmacy of AG with drugs that are metabolized by these enzymes are of concern, especially when using AG in high amounts. The CYP1A2 substrates include anti-psychotics (e.g., olanzapine and clozapine), anesthetics (e.g., lidocaine and zolpidem), antipyretics (e.g., acetaminophen), and cardiovascular drugs (e.g., propranolol) and CYP2C19 substrates include an anti-convulsant (e.g., mephenytoin), anti-acid secretory agents (e.g., omeprazole and pantoprazole), and anti-depressants (e.g., amitriptyline and imipramine). Metabolism of these commonly used drugs at higher rates could result in sub-therapeutic doses. On the other hand, drugs that are metabolically activated by CYP1A2 and CYP2C19, such as antiandrogens (e.g.,

flutamide) and anticoagulants (e.g., clopidogrel) [6,13], might be increasingly biotransformed by AG.

AG extract also induced NAT1 expression. Hence, caution should be warned for combining AG at high amounts (over 120 µg/mL) with NAT1 substrates, such as anti-inflammatory agents (e.g., 5-aminosalicylate (5-AS) [14]. Although NAT1 is not mainly involved in the metabolism of clinical drugs, it has been reported to be involved in carcinogenesis, e.g., bladder, breast, and colorectal cancers, via carcinogen-activating acetylation [7]. Correspondingly, AG extract-induced CYP1A2 expression, and CYP1 has been shown to participate in the bioactivation of procarcinogens to active carcinogens. Expression of CYP1A2 surged in bladder cancer when compared to normal tissues [15]. Consequently, the potential involvement of AG in the development of cancers should be examined.

All concentrations of AG extracts activated CYP2D6, potentially leading to a high risk of HDI when co-administered with CYP2D6 substrates, e.g. anti-histamines (chlorpheniramine, diphenhydramine, and loratadine), anti-cancer agent (tamoxifen), anti-tussive drug (dextromethorphan), anti-depressants (amitriptyline, duloxetine, imipramine, and venlafaxine), anti-nausea (ondansetron), antipsychotics (aripiprazole and risperidone), β-blockers (carvedilol and propranolol), and opioids (codeine and tramadol) [16]. Accordingly, the concomitant use of CYP2D6 substrates and AG should be avoided.

While AG extract did not significantly induce expression of CYP3A4, the predominant clinical drug metabolizing gene, it did affect CYP3A4 expression. Therefore, it is worthwhile to consider the effect of AG on the wide range of CYP3A4 substrates such as immunosuppressants, antibiotics, anti-tumorigenics, anti-hyperlipidemic drugs, anti-hypertensive drugs, anti-hyperglycemic drug, anti-depressants, opioids, and steroids [6].

Ketoconazole is a well-known inhibitor of CYP2C9, CYP2C19, and CYP3A4 enzymes due to its antagonistic effects on constitutive androstane receptor (CAR), glucocorticoid receptor (GR), liver X receptor (LXR), peroxisome proliferator-activated receptor gamma (PPARγ), and farnesoid X receptor (FXR). In contrast, the partial agonistic and agonistic effects of ketoconazole on pregnane X receptor (PXR) and aryl hydrocarbon receptor (AhR) significantly up-regulate the expressions of CYP1A2, CYP3A4, and UGT1A6 [17]. Ketoconazole induced expression of CYP1A2 mRNA in primary human hepatocytes through the AhR regulatory pathway [18] and induced UGT1A6 mRNA expression via PXR and AhR activation [19,20], whilst ketoconazole and rifampicin up-regulated expression of CYP3A4 in primary human hepatocytes via interaction of PXR and hepatocyte nuclear factor 4α (HNF4α) [17,21]. However, regulation of the CYP2D6 and NAT1 transcriptional pathways have been inadequately studied.

From the present results, it appears that AG extract induces CYP1A2, CYP2C19, and NAT1 mRNA expression through the estrogen receptor α (ERα) pathway. Various reports have noted ERα as the transcriptional factor associated with regulation of the expressions CYP1A2, CYP2C19, and NAT1 [6,7], and ERα is not involved in regulating expression of CYP3A4 and UGT1A6. Thus, risk of HDI from AG could be minimized by avoiding polypharmacy of AG with ERα-mediated compound(s).

## CONCLUSION

Up-regulation of phase I CYP450s genes, namely, CYP1A2, CYP2C19, and CYP2D6, and the phase II transferase NAT1 by AG extract in HepG2 cells is exhibited at high concentrations (over 120 µg/mL) of AG. Thus, the concomitant use of AG with clinical drug(s) may result in HDIs, particularly after prolonged use at high dose. Consequently, the risks and benefits of consumption of AG with many types of clinical drugs should be seriously considered. Furthermore, clinical trials should be conducted with regard to these modulatory effects of AG on transcriptional regulation of metabolizing genes in HepG2 cells.

## DECLARATIONS

***Acknowledgement***

Wachirawit Udomsak sincerely expresses gratitude to Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand for scholarship. The authors genuinely acknowledge Research Group for Pharmaceutical Activities of Natural Products using Pharmaceutical Biotechnology (PANPB), Khon Kaen University for research grant and facilities. Dr. Glenn Borlace, Faculty of Pharmaceutical Sciences, Khon Kaen University for English language assistance.

***Conflict of interest***

No conflict of interest is associated with this study.

### *Contribution of authors*

The authors declare that this work was done by the authors named in this article and that all liabilities pertaining to claims relating to the content of this article will be borne by them. Wachirawit Udomsak performed the experimental work and statistical analysis, prepared the figures and tables, and drafted the manuscript. Waranya Chatuphonprasert planned the experimental work, verified the data, supervised the study, and revised the manuscript. Kanokwan Jarukamjorn designed the study, participated in the conceptual framework and discussion, and revised the manuscript. The manuscript was thoroughly read and approved by all the authors for publication.

### *Open Access*



## REFERENCES

1. *Al-Snafi AE. The pharmacological importance of Anethum graveolens. A review. Int J Pharm Pharm Sci 2014; 6(4): 11–13.*
2. *Haidari F, Zakerkish M, Borazjani F, Angali KA, Foroushani GA. The effects of Anethum graveolens (dill) powder supplementation on clinical and metabolic status in patients with type 2 diabetes. Trials 2020; 21(1): 483. doi: 10.1186/s13063-020-04401-3.*
3. *Bahramikia S, Yazdanparast R. Efficacy of different fractions of Anethum graveolens leaves on serum lipoproteins and serum and liver oxidative status in experimentally induced hypercholesterolaemic rat models. Am J Chin Med 2009; 37(4): 685–699.*
4. *Delaquis PJ, Stanich K, Girard B, Mazza G. Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. Int J Food Microbiol 2002; 74(1-2): 101–109.*
5. *Gopalakrishnan T, Ganapathy S, Veeran V, Namasivayam N. Preventive effect of D-carvone during DMBA induced mouse skin tumorigenesis by modulating xenobiotic metabolism and induction of apoptotic events. Biomed Pharmacother 2019; 111: 178–187.*
6. *Zanger UM, Schwab M. Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacol Ther 2013; 138(1): 103–141.*
7. *Jancova P, Anzenbacher P, Anzenbacherova E. Phase II drug metabolizing enzymes. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2010; 154(2): 103–116.*
8. *Donato MT, Lahoz A, Castell JV, Gómez-Lechón MJ. Cell lines: a tool for in vitro drug metabolism studies. Curr Drug Metab 2008; 9(1): 1–11. doi: 10.2174/138920008783331086.*
9. *Kuncharoenwirat N, Chatuphonprasert W, Jarukamjorn K. Differential impacts of phenol red on benzo[a]pyrene and dexamethasone-modified cytochrome P450s in human cancer cells. Pak J Biol Sci 2021; 24(7): 790–800.*
10. *Tatiya-aphiradee N, Chatuphonprasert W, Jarukamjorn K. Ethanolic Garcinia mangostana extract and α-mangostin improve dextran sulfate sodium-induced ulcerative colitis via the suppression of inflammatory and oxidative responses in ICR mice. J Ethnopharmacol 2021; 265: 113384. doi: 10.1016/j.jep.2020.113384. Epub 2020 Sep 11.*
11. *Kuncharoenwirat N, Chatuphonprasert W, Jarukamjorn K. Effects of phenol red on rifampicin-induced expression of cytochrome P450s enzymes. Pharmacophore 2020; 11(3): 13–20.*
12. *Sukkasem N, Chatuphonprasert W, Jarukamjorn K. Hesperidin and myricetin attenuated non-alcoholic fatty liver disease (NAFLD) in HepG2 cells. Trop J Nat Prod Res 2020; 4(10): 739–747.*
13. *Kang P, Dalvie D, Smith E, Zhou S, Deese A, Nieman JA. Bioactivation of flutamide metabolites by human liver microsomes. Drug Metab Dispos 2008; 36(7): 1425–1437.*
14. *Sim E, Abuhammad A, Ryan A. Arylamine N-acetyltransferases: from drug metabolism and pharmacogenetics to drug discovery. Br J Pharmacol 2014; 171(11): 2705–2725.*
15. *Go RE, Hwang KA, Choi KC. Cytochrome P450 1 family and cancers. J Steroid Biochem Mol Biol 2015; 147: 24–30.*
16. *Zanger UM, Turpeinen M, Klein K, Schwab M. Functional pharmacogenetics/genomics of human cytochromes P450 involved in drug biotransformation. Anal Bioanal Chem 2008; 392(6): 1093–1108.*
17. *Novotná A, Krasulová K, Bartoňková I, Korhoňová M, Bachleda P, Anzenbacher P, Dvořák Z. Dual effects of ketoconazole cis-enantiomers on CYP3A4 in human hepatocytes and HepG2 cells. PLoS One 2014; 9(10): e111286. doi: 10.1371/journal.pone.0111286.*
18. *Novotná A, Korhoňová M, Bartoňková I, Soshilov AA, Denison MS, Bogdanova K, Kolar M, Bednar P, Dvořák Z. Enantiospecific effects of ketoconazole on aryl hydrocarbon receptor. PLoS One 2014; 9(7): e101832. doi: 10.1371/journal.pone.0101832.*
19. *Munzel PA, Schmohl S, Buckler F, Jaehrling J, Raschko FT, Köhle C, Bock KW. Contribution of the Ah receptor*

to the phenolic antioxidant-mediated expression of human and rat UDP-glucuronosyltransferase UGT1A6 in Caco-2 and rat hepatoma 5L cells. Biochem Pharmacol 2003; 66(5): 841–847.

20. Xie W, Yeuh MF, Pandya RA, Saini SPS, Negishi Y, Bottroff BS, Cabrera GY, Tukey RH, Evans RM. Control of steroid, heme, and carcinogen metabolism by nuclear pregnane X receptor and constitutive androstane receptor. Proc Natl Acad Sci U S A 2003; 100(7): 4150–4155.
21. Li T, Chiang JYL. Rifampicin induction of CYP3A4 requires PXR crosstalk with HNF4α and co-activators, and suppression of SHP gene expression. Drug Metab Dispos 2006; 34(5): 756–764.