Vol. 14, No. 3, 2023
ISSN 2233-4203/ e-ISSN 2093-8950
ARTICLE www.msletters.org | Mass Spectrometry Letters

Evaluation of the inhibitory effect of *Gynostemma pentaphyllum* extracts on CYP450 enzyme activities using LC-MS/MS

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Received September 28, 2023, Revised September 29, 2023, Accepted September 29, 2023
First published on the web September 30, 2023; DOI: 10.5478/MSL.2023.14.3.116

Abstract : *Gynostemma pentaphyllum* (Thunb.) Makino extract, a natural product with a history of traditional use, has gained attention for its potential health benefits. This study aimed to investigate its effects on key cytochrome P450 (CYP) enzymes using LC-MS/MS. Human liver microsomes and cDNA-expressed CYP2C8, CYP2C9, CYP2C19, and CYP3A4 supersomes were employed. Enzyme activity was assessed based on the formation of CYP-specific marker metabolites. The resulting data showed that the extract exhibited inhibitory effects on CYP2C8, CYP2C9, CYP2C19, and CYP3A4. Thus, *G. pentaphyllum* extract may influence the pharmacokinetics of drugs metabolized by CYP2C8, CYP2C9, CYP2C19, and CYP3A4. These findings emphasize the importance of considering potential herb-drug interactions when incorporating this extract into therapeutic regimens or dietary supplements.

Keywords : *Gynostemma pentaphyllum,* cytochrome P450, herb-drug interaction, LC-MS/MS

Introduction

Gynostemma pentaphyllum (Thunb.) Makino, commonly referred to as *Gynostemma Herba* is a member of the Cucurbitaceae family that thrives in mountainous and forested regions. In Asian countries such as China and Korea, it has a history of use as a substitute for ginseng in traditional remedies. The primary bioactive constituents of *G. pentaphyllum* are gypenosides, a class of saponins, with over 100 distinct species identified. Among these, *G. pentaphyllum* is the most widely distributed and extensively studied due to its composition and potential therapeutic properties.[1,2]

Extensive research has explored the diverse physiological activities associated with gypenosides, highlighting their antioxidative effects, cardiovascular benefits, cholesterol-lowering properties, and hepatoprotective functions.[3-5] More recently, investigations into the immunomodulatory properties of *G. pentaphyllum* components have gained attention.[6] Oral administration of *G. pentaphyllum* tea has been shown to restore diminished splenocyte proliferative capacity caused by cadmium exposure.[7] Additionally, *G. pentaphyllum* extract has demonstrated induced hair growth and an anti-graying effect via complex mechanism related tyrosinase and TRP-2 proteins.[8]

Given the rising popularity of herbal remedies and the use of *G. pentaphyllum* extract in dietary supplements, it is imperative to examine its potential interactions with conventional medications. However, there is a dearth of research investigating the impact of *G. pentaphyllum* extract on CYP-mediated drug metabolism. Cytochrome P450 (CYP) enzymes play a pivotal role in drug metabolism, affecting the bioavailability and efficacy of many pharmaceutical agents.[9] Interactions between herbal products and CYP enzymes can lead to altered drug concentrations in the body, potentially compromising therapeutic outcomes or causing adverse effects. Therefore, this study seeks to address a critical knowledge gap by assessing the potential for drug interactions involving *G. pentaphyllum*. Specifically, we aim to elucidate its inhibitory effects on CYP enzyme activities, shedding light on its potential impact on drug metabolism and highlighting the importance of understanding these interactions for both healthcare professionals and consumers.

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Materials and methods

Chemicals

The *G. pentaphyllum* extract (Lot no. KPM023-026) was obtained from Natural Product Central Bank at the Korea Research Institute of Bioscience and Biotechnology (Daejeon, Korea). We procured pooled human liver microsomes and cDNA-expressed human CYP2C8, CYP2C9, CYP2C19, and CYP3A4 supersomes from BD Gentest (Woburn, MA, USA). Additionally, we acquired the following chemicals from Sigma Aldrich Co.: glucose 6-phosphate, β-$NADP^+$, glucose 6-phosphate dehydrogenase, phenacetin, 7-OH-Coumarin, bupropion, paclitaxel, 4′-OH-diclofenac, 4′-OH-mephenytoin, dextromethorphan, 1′-OH-midazolam, 6′-OH-testosterone, and terfenadine. All other chemicals used were of analytical grade and utilized without further modification. Distilled water was prepared using a Milli-Q purification system (Millipore, Billerica, MA, USA). Prior to use, all standard solutions and mobile phases were passed through a 0.22 μm membrane filter.

CYP inhibition assay

Basic method of CYP inhibition assay was performed as reference.[10] Briefly, *G. pentaphyllum* extract in MeOH (1, 3, 10, 30, 100, and 300 μg/mL) was incubated with a substrate mixture (phenacetin for CYP1A2; coumarin for CYP2A6; bupropion for CYP2B6; paclitaxel for CYP2C8; diclofenac for CYP2C9; 4′-OH -mephenytoin for CYP2C19; dextromethorphan for CYP2D6; and midazolam and testosterone for CYP3A4; Table S1) in potassium phosphate buffer (0.1 M, pH 7.4) with 0.5 mg/mL of human liver microsomes and NADPH-generating system (NGS; 0.1 M glucose-6-phosphate, 10 mg/mL β-$NADP^+$, and 1 U/mL glucose-6-phosphate dehydrogenase) for 30 min in a water bath at 37°C. After incubation, the reaction was stopped by adding 50 μL of internal standard (terfenadine, 0.16 μM) in 1% formic acid in ACN. As for CYP2C8, CYP2C9, CYP2C19, and CYP3A4, each corresponding supersomes and a single substrate (paclitaxel, diclofenac, mephenytoin, midazolam, or testosterone) were used instead of human liver microsomes and the substrate mixture and the inhibitory activity was additionally evaluated.

LC-MS/MS Analysis

An Agilent 1260 binary pump HPLC system, with the Agilent 6460 triple quadrupole mass spectrometer (Agilent Technologies, Palo Alto, CA, USA), equipped with an electrospray ionization (ESI) source, were used as an LC-MS/MS system. Chromatographic separation was achieved on a Fortis C8 column (2.1 × 10 mm, 5.0 μm; Fortis Technologies Ltd, Cheshire, England, UK). The HPLC mobile phases consisted of (A) 0.1 % formic acid and (B) 0.1 % formic acid in 90% acetonitrile. A gradient elution was used with an initial concentration of 15% of solvent B and a flow rate of 0.25 mL/min. The solvent B composition changed as follows: 0–3.0 min, 85% (gradually increased); 3.0–4.5 min, 85% (maintained); 4.5–4.6 min 15%; 4.6–8.0 min, 15% (re-equilibrium). The total run time was 8.0 min, and the injection volume was 5 μL. Mass detection was performed in positive ion mode with multiple reaction monitoring (MRM). The MRM mode used for the precursor-production pairs (Q1/Q3) is shown in Table S2.

Results

Effects of *G. pentaphyllum* Extracts on CYP Enzymatic Activities in Human Liver Microsomes

To assess the impact of *G. pentaphyllum* extracts on *in vitro* human CYP enzymatic activity, we measured the activity of specific marker substrates after treatment with various concentrations of the extract in human liver microsomes. We utilized well-established substrates for CYP isozymes and quantified each metabolite using a product ion monitoring method with LC/MS/MS (Table S2). Since each substrate is metabolized exclusively by a single CYP isozyme when incubated with human liver microsomes, this cassette analysis method enabled us to simultaneously measure the activity of eight substrates. Based on this CYP inhibition test system, we evaluated the potential of *G. pentaphyllum* extracts on *in vitro* human CYP enzymatic activity. Subsequently, the IC_{50} values for CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 were calculated and are presented in Table 1. *G. pentaphyllum* extracts showed inhibitory activities on CYP2C8, CYP2C9, CYP2C19, and CYP3A4 (6-beta-OH-Testosterone). The IC_{50} values were determined to be 75.4 μg/mL, 264.2 μg/mL, 178.5 μg/mL, and 220.6 μg/mL for CYP2C8, CYP2C9, CYP2C19, and CYP3A4 (6-beta-OH-Testosterone), respectively.

Effect of *G. pentaphyllum* Extracts on cDNA-Expressed CYP2C8, CYP2C9, CYP2C19, and CYP3A4

Given the observed inhibitory effect of *G. pentaphyllum* extract on CYP2C8, CYP2C9, CYP2C19, and CYP3A4 activities, we further examined its impact on CYP inhibition using CYP2C8, CYP2C9, CYP2C19, and CYP3A4 supersomes. The formation of metabolites (CYP2C8: Paclitaxel 6-Hydroxylation, CYP2C9: Diclofenac 4-Hydroxylation, CYP2C19: Mephenytoin 4-Hydroxylation, CYP3A4: Midazolam 1-Hydroxylation, and CYP3A4: 6-Beta-OH-Testosterone) at varying concentrations of *G. pentaphyllum* extract is illustrated in Figure 1. The calculated IC_{50} values for CYP2C8, CYP2C9, CYP2C19, CYP3A4 (Midazolam 1-Hydroxylation), and CYP3A4 (6-Beta-OH-Testosterone) were found to be 22.7 μg/mL, 90.8 μg/mL, 125.7 μg/mL, 27.0 μg/mL, and 21.2 μg/mL, respectively.

Discussion

The results of this study clearly demonstrate the inhibitory effects of *G. pentaphyllum* extracts on CYP2C8, CYP2C9,

Table 1. Effects of *G. pentaphyllum* extract on CYP-specific metabolite formation in human liver microsomes.

P450 isozyme	Remaining activities (% of control, n=2)						IC_{50}
	G. pentaphyllum (μg/mL)						
	1	3	10	30	100	300	
CYP1A2 (Phenacetin O-deethylation)	91.1	87.5	85.1	97.4	78.4	62.3	>300
CYP2A6 (Coumarin 7-hydroxylation)	103.6	99.7	98.4	104.6	84.6	66.0	>300
CYP2B6 (hydroxy bupropion)	101.7	101.5	100.2	105.9	76.4	58.9	>300
CYP2C8 (Paclitaxel 6-hydroxylation)	102.0	99.6	94.4	82.8	44.7	19.3	75.4
CYP2C9 (Diclofenac 4-hydroxylation)	99.6	99.4	99.6	107.5	75.1	46.5	264.2
CYP2C19 (Mephenytoin 4-hydroxylation)	112.3	110.2	100.5	103.5	66.6	33.0	178.5
CYP2D6 (Dextromethorphan O-demethylation)	108.9	106.2	105.6	114.0	94.5	69.2	>300
CYP3A4 (Midazolam 1-hydroxylation)	103.3	100.6	97.4	101.4	77.2	52.5	>300
CYP3A4 (6-beta-OH-testosterone)	104.2	101.2	99.0	102.1	71.6	40.2	220.6

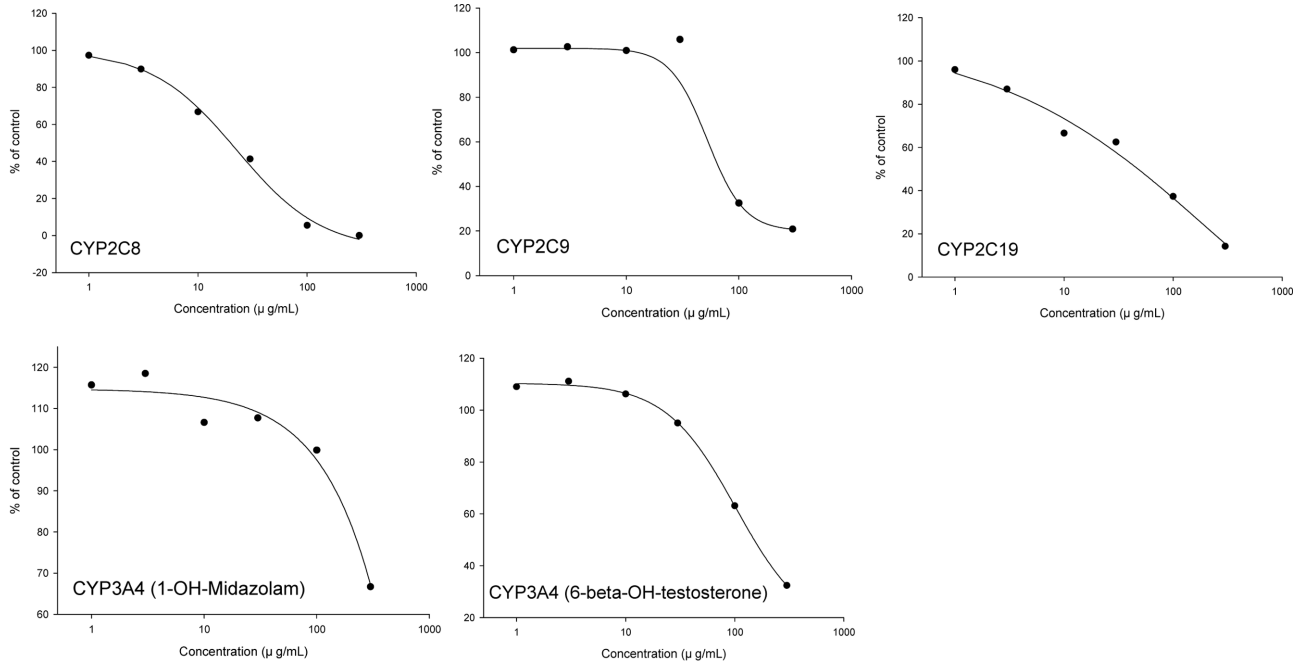

Figure 1. The effect of *G. pentaphyllum* (Thunb.) Makino extract on metabolic activities of CYP2C8, 2C9, 2C19, 3A4. *G. pentaphyllum* (Thunb.) Makino extract was incubated in human CYP supersomes with substrates in the presence of NADPH-generating system for 30 min. The formations of the each CYP enzyme-specific metabolite were plotted as the percentage of control. The IC_{50} value was calculated based on the metabolite formation curves using SigmaPlot 12.0.

CYP2C19, and CYP3A4, all of which are key enzymes involved in drug metabolism. These findings have significant clinical implications, particularly in the context of herb-drug interactions. Several pharmaceutical agents that

interact with these CYP enzymes are currently used in various medical applications. For example, Paclitaxel, an inhibitor of CYP2C8, is employed in the treatment of multiple types of cancer.[11] Diclofenac, an inhibitor of CYP2C9, serves as a non-steroidal anti-inflammatory drug.[12] Mephenytoin, an inhibitor of CYP2C19, is used as an antihistamine.[13] Moreover, Midazolam and testosterone, substrates of CYP3A4, play essential roles in anesthesia development and male reproductive tissue function, respectively.[14]

He et al. reported that gypenosides, known as bioactive components of *G. pentaphyllum* extracts, exhibited competitive inhibition on CYP2D6.[15] However, in our study with *G. pentaphyllum* extracts, the effect on CYP2D6 was not significant. This suggests that the major compounds responsible for the CYP inhibition of *G. pentaphyllum* extract may not be gypenosides.

Considering the findings in this study, it is evident that *G. pentaphyllum* extract may influence the pharmacokinetics and efficacy of drugs metabolized by CYP2C8, CYP2C9, CYP2C19, and CYP3A4. This impact could extend to a wide range of medications, including those used for hyperlipidemia, cardiovascular conditions, herbal medicines, health foods, and teas. Consequently, healthcare professionals and consumers should be aware of the potential for these interactions and exercise caution when combining *G. pentaphyllum* extract with such medications.

In conclusion, this study shows the interactions between *G. pentaphyllum* extract and critical drug-metabolizing enzymes, highlighting the need for further research to elucidate the full scope of these interactions and their clinical implications.

Supporting Information

Supplementary information is available at https://docs.google.com/document/d/1M7qqoXIcH5SRnWstmtNH5ROKxR6Ouu8i/edit?usp=sharing&ouid=111353140014732050956&rtpof=true&sd=true

Acknowledgments

This research was supported by National Research Foundation of Korea (NRF) grants funded by the Ministry of Education (2021R1A2C1010428).

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