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ORIGINAL ARTICLE

Evaluation of Herb–Drug Interactions of *Hovenia dulcis* Fruit Extracts

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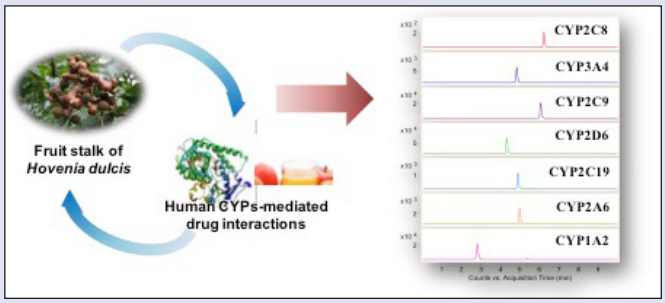
ABSTRACT

Background: *Hovenia dulcis* (Rhamnaceae) fruits are popularly used as herbal medicines or dietary supplements in Asian countries due to functions such as liver protection and detoxification from alcohol poisoning. Accordingly, it is very likely for dietary supplemental products, including *H. dulcis* fruit extracts, to be taken with prescription drugs. **Objective:** In this study, possible food–drug interactions involving *H. dulcis* fruit extracts were evaluated based on the inhibition of cytochrome P450 (CYP) enzyme activity. **Material and Methods:** The water extract of *H. dulcis* fruit extracts was incubated in human liver microsomes with CYP-specific substrates. The formation of the CYP-specific metabolites was measured using liquid chromatography-tandem mass spectrometry. **Results:** *H. dulcis* fruit extracts showed negligible effects on seven CYP isozyme activities at all concentrations tested. **Conclusion:** This result suggests that *H. dulcis* fruit extracts may have minimal pharmacokinetic interactions with coadministered drugs through the modulation of CYP enzymes.

Key words: *Hovenia dulcis*, Herb–drug interactions, CYP450, inhibition

SUMMARY

- Food-drug interactions involving *H. dulcis* fruit extracts were evaluated.
- The inhibition of CYPs by *H. dulcis* extracts was tested.
- *H. dulcis* extracts showed negligible effects on CYP activities.
- *H. dulcis* extracts may have minimal pharmacokinetic interactions with co-administered drugs.



Abbreviations Used: CYP: cytochrome P450 enzymes, HPLC: High performance liquid chromatography, LC-MS/MS : liquid chromatography-tandem mass spectrometry, MRM: multiple-reaction monitoring

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INTRODUCTION

Herbal dietary supplements are receiving remarkable attention worldwide. Such products have traditionally been used as folk remedies and are liable to be regarded as safe.[1] Herbal products are widely used to treat diseases and to improve individuals' health in combination with prescription medicines.[2] However, they are unregulated and many patients do not tell their physicians that they are taking herbal products. Therefore, there is growing concern about unexpected adverse effects induced by herb–drug interactions.[3,4] One of the representative mechanisms of herb–drug interactions are alterations in the absorption and disposition of drugs via the modification of pharmacokinetic regulators, such as cytochrome P450 (CYP) enzymes.[5] CYP enzymes are the major enzymes in the liver, which is a crucial organ for the drug metabolism of many conventional medicines.[6,7] Many herbs could affect the enzyme activity of CYP, which may cause alterations in the metabolic clearance of the substrate drug by inhibiting or inducing a specific CYP enzyme; these modifications may lead to decreased therapeutic effects or increased toxicity of certain drugs.[8-11] For these reasons, assessments of herb–drug interactions involving CYP inhibition based on *in vitro* data should be executed, in order to prevent adverse effects caused by taking prescription medicines in combination with herbal products.

Hovenia dulcis Thunberg belongs to a small genus of *Rhamnaceae* that occurs naturally in East Asia.[12] This tree has been widely used as a traditional herb in Oriental medicine for many years, and the fruit stalk of *H. dulcis* is the main part of the tree that is used for herbal materials.[12,13] The fruit stalks of *H. dulcis* have shown a variety of benefits, including alcohol detoxification,[14,15] hepatoprotective effects,[16,17] antioxidant effects,[18] and antidiabetic effects.[19,20] Therefore, the fruit stalk of *H. dulcis* has been popularly used as an herbal dietary supplement in Asian countries.[21-24] *H. dulcis* products are mainly consumed as drinks, so it is very likely that they may be taken along with prescription or over-the-counter drugs. However, to our knowledge, information on the herb–drug interactions of *H. dulcis* fruit extracts has not yet been reported. Therefore, this study investigated the effects of fruit stalk extracts of *H. dulcis* on CYP-mediated drug metabolism using human liver microsomes, in order to assess and predict herb–drug interactions.

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Table 1: CYP-specific marker substrates and their metabolites monitored

CYP450 isozyme	Marker substrates	Concentration (μM)	Metabolites monitored	MRM ion transition
CYP 1A2	Phenacetin	40	Acetaminophen	152.1→110.1
CYP 2A6	Coumarin	2.5	7-OH-Coumarin	162.9→106.9
CYP 2C8	Paclitaxel	10	6-OH-Paclitaxel	870.4→286.1
CYP 2C9	Diclofenac	10	4-OH-Diclofenac	312.2→230.9
CYP 2C19	(±)-Mephenytoin	80	4-OH-Mephenytoin	235.0→150.1
CYP 2D6	Dextromethorphan	5	Dextrorphan	258.4→157.1
CYP 3A4	Midazolam	2.5	1-OH-Midazolam	343.1→325.1
	Internal standard		Terfenadine	472.4→436.4

MATERIALS AND METHODS

Materials

Aqueous extract samples of fruit stalks of *H. dulcis* were provided by Lifetree Biotech Co., Ltd. (Suwon, Korea). The extract was standardized to contain 0.88 ± 0.05 mg/g of ampelopsin and 0.86 ± 0.05 mg/g of taxifolin by HPLC analysis [Figure 1]. Pooled human liver microsomes were purchased from BD Gentest (Woburn, MA, USA). Glucose-6-phosphate, β-$NADP^+$, glucose-6-phosphate dehydrogenase, phenacetin, coumarin, paclitaxel, diclofenac, mephenytoin, dextromethorphan, midazolam, furafylline, methoxsalen, sulfaphenazole, ticlopidine, quercetin, quinidine, ketoconazole, and terfenadine were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Solvents for HPLC were purchased from J. T. Baker (Phillipsburg, NJ, USA). Distilled water was prepared using a MilliQ purification system (Millipore, Billerica, MA, USA).

CYP inhibition assay

The CYP inhibition assay was performed according to the previously established method.[25] Various concentrations of fruit stalk extracts from *H. dulcis* (1, 3, 10, 30, and 100 μg/mL in 70% methanol) were tested without and with a preincubation procedure, to test the possibility of mechanism-based inactivation. Well-known selective CYP inhibitors were used as positive controls. The inhibitors and their concentrations were as follows: 10 μM of furafylline for CYP1A2; 20 μM of methoxsalen for CYP2A6; 10 μM of quercetin for CYP2C8; 50 μM of sulfaphenazole for CYP2C9; 20 μM of ticlopidine for CYP2C19; 50 μM of quinidine for CYP2D6; and 5 μM of ketoconazole for CYP3A4. The resultant incubation samples were pretreated using solid-phase extraction, then analyzed based on multiple-reaction monitoring (MRM) detection using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The CYP specific marker substrates used, and the metabolites monitored with their MRM precursor-product transition conditions, are tabulated in Table 1.

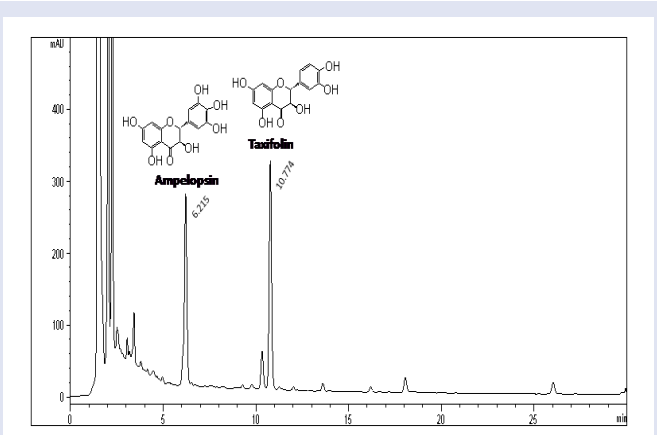

Figure 1: Representative HPLC chromatogram of *H. dulcis* fruit extracts. An Xbridge™ Shield RP18 column (4.6 mm I.D. × 150 mm, 3.5 μm) (Waters, Milford, USA) was used for the chromatographic separation. Column oven temperature maintained at 30°C. The mobile phase was composed of 0.1% acetic acid (solvent A) and acetonitrile (solvent B). The flow rate was 1.0 mL/min. The composition of solvent B was maintained at 20% for 3 min, linearly increased to 38% for 24 min, further increased to 90% for 1 min and maintained at 90% for 5 min, which was followed by equilibration to the initial composition for 6 min. The injection volume was 10 μL and the UV detection wavelength was 290 nm.

High performance liquid chromatography (HPLC) analysis

The content of the major bioactive constituents of *H. dulcis*, ampelopsin, and taxifolin was measured in the extract sample using HPLC. The HPLC analysis was performed based on the previously reported method.[26] The extract sample was dissolved in 80% methanol at a concentration of 250 mg/mL, filtered, and injected into the HPLC system. Ampelopsin and taxifolin were eluted at 6.2 and 10.8 min, respectively [Figure 1].

RESULTS AND DISCUSSION

The inhibitory effects of fruit stalk extract of *H. dulcis* were examined in human liver microsomes. The validity of the CYP assay system was assessed with well-known selective inhibitors of CYP isozymes: Furafylline (CYP1A2), methoxsalen (CYP2A6), quercetin (CYP2C8), sulfaphenazole (CYP2C9), ticlopidine (CYP2C19), quinidine (CYP2D6), and ketoconazole (CYP3A4). Each inhibitor selectively inhibited the corresponding CYP marker activity. The remaining activity of the CYP inhibitors was as follows: 2.79% for CYP1A2 (furafylline), 1.74% for CYP2A6 (methoxsalen), 6.64% for CYP2C8 (quercetin), 3.76% for CYP2C9 (sulfaphenazole), 16.57% for CYP2C19 (ticlopidine), 1.65% for CYP2D6 (quinidine), and 2.79% for CYP3A4 (ketoconazole) at designated concentrations. Tables 2 and 3 show the effect of fruit stalk

Table 2: Remaining activities of CYP isozymes following treatment with *H. dulcis* extracts (without preincubation)

CYP450 isozyme	Remaining activities (% of control)*					IC_{50} (μg/mL)
	H. dulcis extract (μg/mL)					
	1	3	10	30	100	
CYP 1A2	107.0	99.1	90.9	93.0	96.6	>100
CYP 2A6	83.3	90.6	94.9	101.5	99.4	>100
CYP 2C8	97.6	92.2	80.1	112.1	82.6	>100
CYP 2C9	99.9	101.3	89.5	106.8	105.0	>100
CYP 2C19	81.2	89.2	87.5	83.6	105.2	>100
CYP 2D6	99.8	94.3	82.8	100.7	101.4	>100
CYP 3A4	90.9	98.0	87.8	103.6	102.3	>100

*Data are shown as mean remaining activity (% of control) of duplicate measurements.

Table 3: Remaining activities of CYP isozymes following treatment with *H. dulcis* extracts (with preincubation)

CYP450 isozyme	Remaining activities (% of control)*					
	H. dulcis extract (μg/mL)					IC50 (μg/mL)
	1	3	10	30	100	
CYP 1A2	128.3	105.0	101.2	86.1	114.7	>100
CYP 2A6	116.7	119.1	115.0	95.0	91.7	>100
CYP 2C8	87.9	81.1	94.5	83.3	78.1	>100
CYP 2C9	92.0	97.1	107.6	94.9	96.0	>100
CYP 2C19	105.1	97.9	97.0	83.4	91.8	>100
CYP 2D6	90.8	102.7	106.3	96.8	91.7	>100
CYP 3A4	99.0	99.4	117.4	87.8	97.8	>100

*Data are shown as mean remaining activity (% of control) of duplicate measurements.

extracts of *H. dulcis* on the drug-metabolizing activity of seven CYP isozymes. When fruit stalk extracts of *H. dulcis* were tested without preincubation, no significant inhibitory effects on CYP isozyme enzyme activities were observed at any of the concentrations tested [Table 2]. When the fruit stalk extracts of *H. dulcis* were preincubated and tested, the extent of inhibition on several CYP isozyme activities was slightly different from the results shown in [Table 3], but no inhibitory effects on CYP isozyme enzyme activities were observed, and the IC_{50} values were all >100 μg/mL [Table 3]. Therefore, fruit stalk extracts of *H. dulcis* are considered to have negligible effects on drug metabolism regulated by CYP isozymes as competitive or mechanism-based inhibitors.

There have been several published reports demonstrating the CYP 450 inhibitory effects caused by the major bioactive compounds of *H. dulcis* extracts, ampelopsin, and taxifolin. Huang *et al.*[27] reported that ampelopsin significantly inhibited CYP2C9 activity and induced CYP3A4 activity after oral administration in rats. Çelik *et al.*[28] showed that taxifolin acts as a weak inhibitor of cytochrome b5 reductase, which is involved in specific CYP450-mediated drug metabolism by donating a second electron to CYP450 cytochrome b5. These previous reports raised some concerns about the possibility of inhibited CYP enzyme activity by *H. dulcis* extracts. However, in this study, no considerable inhibitory effects of *H. dulcis* extracts on CYP 450 enzymes were observed. It is supposed that the chemical complexity of *H. dulcis* extracts may compensate for or dilute the effects of ampelopsin and taxifolin. The representative constituents of *H. dulcis* extracts are phenolic compounds and triterpene saponins.[26] As phenolic compounds, hovenodulinol, hovenitins I, II, and III, (+)-3,3',5',5,7-pentahydroflavanone, laricitrin, myricetin, (+)-gallocatechin, dihydrokaempferol, dihydromyricetin (ampelopsin), and quercetin have been reported. Saponin C2, β-daucosterol, hovenidulciosides A1, A2, B1, and B2, hodulosides I and III, and hovenidulcigenin have been reported as triterpene saponins. The property of each compound on CYP inhibition has not yet been fully elucidated. However, considering the content of ampelopsin and taxifolin in the extract (less than 0.1%), the effect on CYP activity by these compounds in the extract is expected to be negligible as observed in this study.

CONCLUSION

The potential effects of fruit stalk extracts of *H. dulcis* on human CYP enzyme activities were evaluated *in vitro*, and the results showed a possible negligible interaction with coadministered drugs by the modulation of CYP enzymes. However, differences may exist between *in vitro* and *in vivo* test results. In addition, there could be other herb–drug interaction mechanisms involved with *H. dulcis* extracts and continued and further studies of such interactions should be conducted.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Ziment I, Tashkin DP. Alternative medicine for allergy and asthma. J Allergy Clin Immunol 2000;106:603-14.
2. Wang J, Yang X, Feng B, Qian W, Fang Z, Liu W, *et al.* Is Yangxue Qingnao Granule combined with antihypertensive drugs, a new integrative medicine therapy, more effective than antihypertensive therapy alone in treating essential hypertension? Evid Based Complement Alternat Med 2013 2013:540-613.
3. Fasinu PS, Bouic PJ, Rosenkranz B. An overview of the evidence and mechanisms of herb-drug interactions. Front Pharmacol 2012;3:1-19.
4. Gouws C, Steyn D, Du Plessis L, Steenekamp J, Hamman JH. Combination therapy of Western drugs and herbal medicines: recent advances in understanding interactions involving metabolism and efflux. Expert Opin Drug Metab Toxicol 2012;8:973-84.
5. Sprouse AA, van Breemen RB. Pharmacokinetic interactions between drugs and botanical dietary supplements. Drug Metab Dispos 2016;44:162-71.
6. Gervasini G, Caballero MJ, Carrillo JA, Benitez J. Comparative cytochrome p450 *in vitro* inhibition by atypical antipsychotic drugs. ISRN Pharmacol 2013;2013:792456.
7. Kim J, Coss CC, Barrett CM, Mohler ML, Bohl CE, Li CM. Role and pharmacologic significance of cytochrome P-450 2D6 in oxidative metabolism of toremifene and tamoxifen. Int J Cancer 2013;132:1475-85.
8. Ueng YF, Ko HC, Chen CF, Wang JJ, Chen KT. Modulation of drug-metabolizing enzymes by extracts of a herbal medicine *Evodia rutaecarpa* in C57BL/6J mice. Life Sci 2002;71:1267-77.
9. Izzo AA, Di Carlo G, Borrelli F, Ernst E. Cardiovascular pharmacotherapy and herbal medicines: the risk of drug interaction. Int J Cardiol 2005;98:1-14.
10. Liu KH, Kim MJ, Jeon BH, Shon JH, Cha IJ, Cho KH. *et al.* Inhibition of human cytochrome P450 isoforms and NADPH-CYP reductase *in vitro* by 15 herbal medicines, including *Epimedii herba.* J Clin Pharm Ther 2006;31:83-91.
11. Gurley BJ, Swain A, Hubbard MA, Williams DK, Barone G, Hartsfield F. *et al.* Clinical assessment of CYP2D6-mediated herb–drug interactions in humans: Effects of milk thistle, black cohosh, goldenseal, kava kava, St. John's wort, and *Echinacea.* Mol Nutr Food Res 2008;52:755-63.
12. Lim TK. *Hovenia dulcis* Edible medicinal and non-medicinal plants. Netherlands: Springer; 2013.pp. 568-77.
13. Hyun TK, Eom SH, Yu CY, Rotisch T. *Hovenia dulcis*-an Asian traditional herb. Planta Med 2010;76:943-9.
14. Yoshikawa M, Murakami T, Ueda T, Yoshizumi S, Ninomiya K, Murakami N. *et al.* Bioactive constituents of Chinese natural medicines. III. Absolute stereostructures of new dihydroflavonols, hovenitins I, II, and III, isolated from hoveniae semen seu fructus, the seed and fruit of Hovenia dulcis THUNB. (Rhamnaceae): inhibitory effect on alcohol-induced muscular relaxation and hepatoprotective activity. Yakugaku Zasshi 1997;117:108-18.
15. Chen SH, Zhong GS, Li AL, Li SH, Wu LK. Influence of *Hovenia dulcis* on alcohol concentration in blood and activity of alcohol dehydrogenase (ADH) of animals after drinking. Zhongguo Zhong Yao Za Zhi 2006;31:1094-6.
16. Wang M, Zhu P, Jiang C, Ma L, Zhang Z, Zeng X. Preliminary characterization, antioxidant activity *in vitro* and hepatoprotective effect on acute alcohol-induced liver injury in mice of polysaccharides from the peduncles of *Hovenia dulcis.* Food Chem Toxicol 2012;50:2946-70.
17. Fang HL, Lin HY, Chan MC, Lin WL, Lin WC. Treatment of chronic liver injuries in mice by oral administration of ethanolic extract of the fruit of *Hovenia dulcis.* Am J Chin Med 2007;35:693-703.
18. Na CS, Yoon SY, Kim JB, Na DS, Dong MS, Lee MY. *et al.* Anti-fatigue activity of *Hovenia dulcis* on a swimming mouse model through the inhibition of stress hormone expression and antioxidation. Am J Chin Med 2013;41:945-55.
19. Ji Y, Chen S, Zhang K, Wang W. Effects of *Hovenia dulcis* Thunb on blood sugar and hepatic glycogen in diabetic mice. Zhong Yao Cai 2002;25:190-1.

20. Wu LH, Zhang J. Evaluation of anti-diabetic activities of *Hovenia dulcis* Thunb. Adv Mat Res 2012;554:1827-30.
21. Xiang J, Zhu W, Li Z, Ling S. Effect of juice and fermented vinegar from *Hovenia dulcis* peduncles on chronically alcohol-induced liver damage in mice. Food Funct 2012;3:628-34.
22. Park EM, Ye EJ, Kim SJ, Choi HI, Bae MJ. Eliminatory effect of health drink containing *Hovenia dulcis* Thunb extract on ethanol-induced hangover in rats. J Korean Soc Food Cult 2006;21:71-5.
23. Oh KH, Song HS. Sensory evaluation of seasoned soy sauce with hutgae (*Hovenia dulcis* Thunb) fruit and pear extracts. Korean J Food Nutr 2013;26:323-8.
24. Won SB, Song HS. Antioxidant activity and sensory evaluation in soy sauce with fruit, stem, or twig of *Hovenia dulcis* thunb. Korean J Food Nutr 2013;26:258-65.
25. Kim IS, Kim Y, Kwak TH, Yoo HH. Effects of β-lapachone, a new anticancer candidate, on cytochrome P450-mediated drug metabolism. Cancer Chemother Pharmacol 2013;72:699-702.
26. Park JS, Kim IS, Rehman SU, Na CS, Yoo HH. HPLC Determination of Bioactive Flavonoids in Hovenia dulcis Fruit Extracts. J Chromatogr Sci 2016;54:130-5.
27. Huang Y, Xu ZS, Ye Q. Effect of dihydromyricetin on cytochrome P450 isoforms CYP1A2, CYP2C9 and CYP3A4 in rats. Lat Am J Pharm 2013;32:1570-4.
28. Çelik H, Koşar M, Arinç E. *In vitro* effects of myricetin, morin, apigenin, (+)-taxifolin, (+)-catechin, (−)-epicatechin, naringenin and naringin on cytochrome b5 reduction by purified NADH-cytochrome b5 reductase. Toxicology 2013;308:34-40.