ABSTRACT

Objective: The aim of this study was to assess the influence of traditional herbal formulas, including Bangpungtongseong-san (BPTSS; Fangfengtongsheng-san, Botu-tsusho-san), Ojeok-san (OJS; Wuijisan, Goshaku-san), and Oyakusngi-san (OYSGS; Wuyaoshungi-san, Uyakujyunki-san), on the activities of the human cytochrome P450s (CYP450s) and UDP-glucuronosyltransferases (UGTs), which are drug-metabolizing enzymes. Materials and Methods: The activities of the major human CYP450 isozymes (CYP1A2, CYP3A4, CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP2E1) and UGTs (UGT1A1, UGT1A4, and UGT2B7) were investigated using in vitro fluorescence-based and luminescence-based enzyme assays, respectively. The inhibitory effects of the herbal formulas were characterized, and their IC₅₀ values were determined. Results: BPTSS inhibited the activities of CYP1A2, CYP2C19, CYP2E1, and UGT1A1 while it exerted relatively weak inhibition on CYP2B6, CYP2C9, CYP2D6, and CYP3A4. BPTSS also negligibly inhibited the activities of UGT1A1 and UGT2B7 with IC₅₀ values in the excess of 1000 μg/mL. OJS and OYSGS inhibited the activity of CYP2D6, whereas they exhibited no inhibition of the UGT1A4 activity at doses <1000 μg/mL. In addition, OJS inhibited the CYP1A2 activity but exerted a relatively weak inhibition on the activities of CYP2C9, CYP2C19, CYP2E1, and CYP3A4. Conversely, OJS negligibly inhibited the activities of CYP2B6, UGT1A1, and UGT2B7 with IC₅₀ values in excess of 1000 μg/mL. OYSGS weakly inhibited the activities of CYP1A2, CYP2C19, CYP2E1, CYP3A4, and UGT1A1, with a negligible inhibition on the activities of CYP2B6, CYP2C9, and CYP2D6, with IC₅₀ values in excess of 1000 μg/mL. Conclusions: These results provide information regarding the safety and effectiveness of BPTSS, OJS, and OYSGS when combined with conventional drugs.

Key words: Cytochrome P450s, herb-drug interactions, traditional herbal formulas, UDP-glucuronosyltransferases

SUMMARY

- Bangpungtongseong-san inhibited the activities of human microsomal CYP1A2, CYP2C19, CYP2E1, and UGT1A1, with a negligibly inhibition on the activities of CYP2B6, CYP2C9, CYP2D6, CYP3A4, UGT1A1, and UGT2B7.
- Ojeok-san (OJS) inhibited the CYP1A2 and CYP2D6 mediated metabolism while showing a comparatively weak inhibition against CYP2B6, CYP2C9, CYP2C19, CYP2E1, and CYP3A4 in human microsomes.

- Oyakusngi-san (OYSGS) inhibited the activities of human microsomal CYP2D6, with a relatively weak inhibition on the activities of CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2E1, CYP3A4, UGT1A1, and UGT2B7.
- OJS showed no inhibition on the activities of human microsomal UGT1A4 and UGT2B7, and OYSGS did not affect the human microsomal UGT1A4 activity.

INTRODUCTION

Drug metabolism is responsible for the biotransformation of xenobiotics, including therapeutic drugs and endogenous/exogenous substances, yielding products that are more soluble in water than are their parent substances. Drug-metabolizing enzymes are classified into two groups, phase I and II enzymes, and cytochrome P450 (CYP450) and UDP-glucuronosyltransferase (UGT) are responsible for the phase I and phase II transformation reactions, respectively.1-4

CYP450s participate in the oxidative metabolism of a variety of xenobiotics. CYP450s consist of numerous families and subfamilies...
In this study, the effects of the traditional herbal formulas BPTSS, OJS, and OYSGS on the activities of the major human CYP450s (CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4), and UGTs (UGT1A1, UGT1A4, and UGT2B7) were investigated in vitro CYP450 isozyme and UGT isozyme assays.

Table 1: The compositions of the three herbal formulas

| Crude drug | Scientific name | Bangpungtongseong-san | Ojeok-san | Oyaksungi-san |
|------------|----------------|-----------------------|-----------|--------------|
| Talcum | Talcum | 6.38 | | |
| Glycyrrhiza Radix et Rhizome | Glycyrrhiza uralensis | 4.50 | 2.20 | 1.13 |
| Gypsum Fibrosum | Gypsum | 2.63 | | |
| Scutellariae Radix | Scutellaria baicalensis | 2.63 | | |
| Platycodonis Radix | Platycodon grandiflorum | 2.63 | 3.00 | 3.75 |
| Saposhnikoviae Radix | Ledebouria seelosiodes | 1.69 | | |
| Paoniae Radix | Paonia lactiflora | 1.69 | 3.00 | |
| Cnidii Rhizoma | Cnidium officinale | 1.69 | 2.60 | 3.75 |
| Angelicae Gigantis Radix | Angelica gigas | 1.69 | 3.00 | |
| Rhei Radix et Rhizoma | Rheum undulatum | 1.69 | | |
| Ephedrae Herba | Ephedra sinica | 1.69 | 3.70 | 5.63 |
| Menthae Herba | Mentha pulegium | 1.69 | | |
| Forsythiae Fructus | Forsythia koreana | 1.69 | | |
| Natrii Sulphas | Natrī sulfās | 1.69 | | |
| Schizonepetae Spica | Schizonepeta tenuifolia | 1.31 | | |
| Atractylodis Rhizoma Alba | Atractylodes japonica | 1.31 | | |
| Gardeniae Fructus | Gardenia jasminoides | 1.31 | | |
| Zingiberis Rhizoma Crudus | Zingiber officinale | 6.25 | 3.70 | 3.75 |
| Atractylodis Rhizoma | Atractylodes lancea | 7.50 | | |
| Citri Unshii Pericarpium | Citrus unshiu | 3.70 | 5.63 | |
| Magnoliae Cortex | Magnolia officinalis | 3.00 | | |
| Angelicae Dahuricae Radix | Angelica dahurica | 2.60 | 3.75 | |
| Aurantii Fructus Immaturus | Citrus unshiu | 3.00 | 3.75 | |
| Zingiberis Rhizoma | Zingiber officinale | 3.00 | 1.88 | |
| Hoelen | Poria cocos | 3.00 | | |
| Pinelliae Tuber | Pinellia ternata | 2.60 | | |
| Cinnamomi Bark | Cinnamomum cassia | 2.60 | | |
| Allii Radix | Allium fistulosum | 3.70 | | |
| Linderae Radix | Lindera strychnifolia | 5.63 | 3.75 | |
| Bombycis Corpus | Bombyx mori | 3.75 | | |
| Zizyphi Fructus | Zizyphus jujuba | 46.15 | | |
| Total amount (g) | 44.16 | 55.90 | 46.15 | |
| Yield (%) | 17.70 | 21.00 | 24.40 | |
MATERIALS AND METHODS

Chemicals and materials

Reference standards, albuflorin, paconflorin, geniposide, liquiritin, baicalin, glycerrhizin, ferulic acid, cinnamaldehyde, naringin, and 6-gergnerol were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Hesperidin and neohesperidin were purchased from Biopurify Phytochemicals (Chengdu, China). Nodakenin was purchased from NPC BioTechnology, Inc., (Daejeon, Korea). The purity of all reference standards was ≥ 98.0%. High-performance liquid chromatography (HPLC) grade methanol, acetonitrile, and water were obtained from J.T. Baker (Phillipsburg, NJ, USA). Glacial acetic acid of analytical reagent grade was purchased from Junsei (Tokyo, Japan).

Vivid CYP450 Screening Kits (Vivid CYP1A2 Blue, Vivid CYP2B6 Blue, Vivid CYP2C9 Blue, Vivid CYP2C19 Blue, Vivid CYP2D6 Blue, Vivid CYP2E1 Blue, and Vivid CYP3A4 Green) were purchased from Invitrogen Co., (Camarillo, CA, USA). These kits use 7-ethoxy-methylxol-3-cyanocoumarin as a substrate for CYP1A2, CYP2B6, CYP2C19, and CYP2E1. In addition, di (benzoxylmethoxy) fluorescein was used as a substrate for CYP3A4, and 7-benzoyl-oxyl-4-trifluoromethylcoumarin was used as a substrate for CYP2B6 and CYP2C9. UGT-Glo UGT1A1 and UGT2B7 Screening Systems were purchased from Promega (Madison, WI, USA). The recombinant human UGT1A4 enzyme was purchased from Corning Inc. Life Science (Tewksbury, MA, USA). α-Naphthoflavone, ketoconazol, miconazol, sulfaphenazol, quinidine, sodium diethyldithiocarbamate trihydrate, dicyclofenac, and lopinavir were obtained from Sigma Chemical Co., (St. Louis, MO, USA). All other chemicals were of analytical grade.

Preparation of herbal formula extracts

The crude herbs forming the herbal formulations of BPTSS, OJS, and OYSGS were purchased from a traditional herb market, Omniherb (Yeongcheon, Korea) and HMAX (Jechon, Korea). All herbs were taxonomically confirmed by Professor Je-Hyeon Lee, Dongguk University, Korea. To obtain the water decoction of the three herbal formulas, each herbal medicine was chopped and mixed as shown in Table 1. The extraction of each herbal formula was performed in distilled water at 100°C for 120 min using an electric extractor (COSMOS-660; Kyungseo Machine Co., Incheon, Korea). The crude herbs forming the herbal formulations of BPTSS, OJS, and OYSGS were chopped and mixed as shown in Table 1. The extraction of each herbal formula was performed in distilled water at 100°C for 120 min using an electric extractor (COSMOS-660; Kyungseo Machine Co., Incheon, Korea). The crude herbs forming the herbal formulations of BPTSS, OJS, and OYSGS were purchased from a traditional herb market, Omniherb (Yeongcheon, Korea) and HMAX (Jechon, Korea). All herbs were taxonomically confirmed by Professor Je-Hyeon Lee, Dongguk University, Korea. To obtain the water decoction of the three herbal formulas, each herbal medicine was chopped and mixed as shown in Table 1. The extraction of each herbal formula was performed in distilled water at 100°C for 120 min using an electric extractor (COSMOS-660; Kyungseo Machine Co., Incheon, Korea).

Life Sciences, Ann Arbor, MI, USA) before HPLC analysis. The assays were performed using the Vivid CYP450 Reaction Buffer and Regeneration System (consisting of glucose-6-phosphate and glucose-6-phosphate dehydrogenase), and the plate was incubated for 20 min to allow the samples to interact with the CYP enzymes. After preincubation, the reaction was started by adding 10 μL of the Vivid Substrate and NADP+. The regeneration system converts NADP+ into NADPH, which is required to start the CYP450 reaction. The enzymatic reaction is initiated by the addition of a mixture of NADP+ and the appropriate Vivid Substrate. The fluorescence intensity was measured using an EnVision2103 Multilabel Reader (PerkinElmer Inc., MA, USA) for 15 min at the excitation and emission wavelengths of 485 and 535 nm, respectively, for CYP3A4. For CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP2E1, the fluorescence intensity was measured for 60 min at the excitation and emission wavelengths of 415 and 460 nm, respectively, using a SpectraMax i3 ( Molecular Devices Co., Sunnyvale, CA, USA).

The inhibition percentage (%) was obtained via the following equation: % Inhibition = 1 - (S1-S0)/(C1-C0) ×100, where C1 is the fluorescence of the control after incubation, C0 is the initial fluorescence of the control, S1 is the fluorescence of the test sample after incubation, and S0 is the initial fluorescence of the test sample in the linear section.

High-performance liquid chromatography analysis

For quality assessment of the three formulas of BPTSS, OJS, and OYSGS, a chromatographic analysis was performed using a Shimadzu Prominance LC-20A series (Kyoto, Japan), equipped with a solvent delivery unit, an on-line degasser, a column oven, an autosampler, and a photo diode array (PDA) detector. The data were acquired and processed using Lc solution software (Version 1.24, Shimadzu Co., Kyoto, Japan). The constituents in each formula were separated on a Phenomenex Gemini C18 column (250 mm × 4.6 mm, 5 μm, Torrance, CA, USA) for OJS and OYSGS and a Phenomenex Luna C18 column (250 mm × 4.6 mm, 5 μm, Torrance, CA, USA) for BPTSS, with the column temperature set to 40°C. The mobile phases consisted of 0.1% (v/v) acetic acid in distilled water (A) and 0.1% (v/v) acetic acid in acetonitrile (B). The gradient elutions of the mobile phases are shown in Table 2. The flow-rate and injection volume were 1.0 mL/min and 10 μL, respectively. For HPLC analysis of each formula, 200, 200 and 400 μg of lyophilized BPTSS, OJS, and OYSGS extract were dissolved in 20 mL of distilled water, respectively, and then, the solution was filtered through a SmartPor GHP 0.2 μm syringe filter (PALL Life Sciences, Ann Arbor, MI, USA) before HPLC analysis.

Cytochrome P450 isozyme assay

The assays were performed using the Vivid CYP450 Screening Kits according to the protocol provided by the manufacturer. The Vivid CYP450 Screening Kits are designed to assess the metabolic activity of the predominant human CYP450 isozymes involved in hepatic drug metabolism: CYP1A2, CYP3A4, CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP2E1. The Vivid Substrate and Fluorescent Standards were reconstituted, and a standard curve was prepared. A test sample of 40 μL diluted in solvent, a positive inhibition control (a compound that inhibits the respective CYP450 enzyme), or a solvent control was added to each well. The solutions were mixed after adding 50 μL of the Master Pre-Mix containing P450 BACULOSOMES in the Vivid CYP450 Reaction Buffer and Regeneration System (consisting of glucose-6-phosphate and glucose-6-phosphate dehydrogenase), and the plate was incubated for 20 min to allow the samples to interact with the CYP enzymes. After preincubation, the reaction was started by adding 10 μL of the Vivid Substrate and NADP+. The regeneration system converts NADP+ into NADPH, which is required to start the CYP450 reaction. The enzymatic reaction is initiated by the addition of a mixture of NADP+ and the appropriate Vivid Substrate. The fluorescence intensity was measured using an EnVision2103 Multilabel Reader (PerkinElmer Inc., MA, USA) for 15 min at the excitation and emission wavelengths of 485 and 535 nm, respectively, for CYP3A4. For CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP2E1, the fluorescence intensity was measured for 60 min at the excitation and emission wavelengths of 415 and 460 nm, respectively, using a SpectraMax i3 (Molecular Devices Co., Sunnyvale, CA, USA).

The inhibition percentage (%) was obtained via the following equation: % Inhibition = 1 - (S1-S0)/(C1-C0) ×100, where C1 is the fluorescence of the control after incubation, C0 is the initial fluorescence of the control, S1 is the fluorescence of the test sample after incubation, and S0 is the initial fluorescence of the test sample in the linear section.

Table 2: Solvent gradient for analysis of high-performance liquid chromatography-photo diode array

| Time (min) | Bangpungtongseong-san | Ojeok-san | Oyak-sungi-san |
|-----------|-----------------------|-----------|---------------|
|           | A (%) | B (%) | A (%) | B (%) | A (%) | B (%) |
| 0         | 95    | 5     | 0     | 85    | 15    | 0     | 85    | 15    |
| 40        | 25    | 70    | 20    | 75    | 25    | 40    | 35    | 65    |
| 45        | 0     | 100   | 40    | 45    | 55    | 45    | 0     | 100   |
| 50        | 0     | 100   | 45    | 0     | 100   | 50    | 0     | 100   |
| 55        | 95    | 5     | 50    | 0     | 100   | 55    | 85    | 15    |
| 70        | 95    | 5     | 55    | 85    | 15    | 70    | 85    | 15    |

a1.0% (v/v) acetic acid in water; b1.0% (v/v) acetic acid in acetonitrile
The background fluorescence of the herbal formulas was corrected by subtracting the values obtained from the incubation without substrates. The CYP450 inhibition of each sample was expressed regarding IC50 as calculated from the log-dose inhibition curve (SigmaPlot, version 12.5, Systat Software, Inc., CA, USA). The data were expressed as the mean ± standard error of the mean (SEM) (n = 3). α-Naphthoflavone, ketoconazole, sulfaphenazole, quinidine, and sodium diethyldithiocarbamate trihydrate were used as positive controls for CYP1A2, CYP3A4, CYP2C9, CYP2D6, and CYP2E1, respectively. Miconazole was used as a positive control for CYP2B6 and CYP2C19.

UDP-glucuronosyltransferase isozyme assay

The assays were performed using the UGT-Glo™ Screening Systems according to the manufacturer's protocol. The assay systems provide a luminescent method for measuring the activity of UGTs. Two glucuronidation reactions were set up in parallel to measure UGT activity. Both reactions contained a source of UGT (UGT1A1, UGT1A4, or UGT2B7) and the procluciferin substrates (UGT multi-enzyme substrate or UGT1A4 substrate), but only one of them contained the uridine 5′-diphosphoglucuronic acid (UDPGA) cofactor. Ten microliters of 4× concentrated test sample; diclofenac, which is a known inhibitor of the UGT1A1 and UGT2B7 isozymes; lopinavir, as a UGT1A4 inhibitor; or vehicle was added each well (white opaque 96 well-plate, Corning Inc., NY, USA). Then, 10 μL of UDPGA (plus-UDPGA reaction set) or distilled water (minus-UDPGA reaction set) was added to the relevant wells. Twenty microliters of the prepared 2× control reaction mixture (minus-UGT enzyme) and 2× UGT reaction mixture (UGT1A1, UGT1A4, or UGT2B7) were added to the appropriate wells. The reaction solution was mixed and incubated at 37°C for 90, 180, or 60 min, respectively, for UGT1A1, UGT1A4, and UGT2B7. The final contents of the reactant were 0.1 mg/mL UGT enzyme and 20 μM enzyme substrate in the presence or absence of 4 mm UDPGA. After incubation, 40 μL of the reconstituted luciferin detection reagent plus D-cysteine was added to all wells. After 20 min of incubation at room temperature, the luminescence signal was detected using a SpectraMax® i3.

The detected data were converted to the calculated the difference using the following percent of substrate consumed (%SC) equation: % Substrate consumed = (background corrected difference)/(average minus-UDPGA values) × 100. The inhibition percentage (%) was obtained via the following equation: % Inhibition = (1 – [S/CAVR]) × 100, where S is the %SC of each sample or the control wells, and CAVR is the average %SC of the control wells. The UGT inhibition of each sample was expressed regarding IC50 as calculated using computer software (SigmaPlot) capable of generating a four parameter logistic curve fit. The data were expressed as the mean ± SEM (n = 2).

**RESULTS**

High-performance liquid chromatography analysis of herbal formulas

The developed HPLC-PDA method was subsequently applied for the quality control of the three formulas of BPTSS, OJS, and OYSGS. Consequently, the marker compounds in BPTSS, OJS, and OYSGS eluted within 40, 45, and 35 min, respectively, and the typical three-dimensional chromatograms are shown in Figure 1. The correlation coefficient (r2) of all analytes showed good linearity (≥0.9997). Using the optimized chromatography conditions, the amounts of the various marker compounds in BPTSS, OJS, and OYSGS are summarized in Table 3.

| Compound     | Contents in extract (mg/g) |
|--------------|----------------------------|
|              | Bangpungtongseong-san | Ojeok-san | Oyaksungi-san |
| Geniposide   | 5.59±0.012             |           |               |
| Baicalin     | 13.53±0.120            |           |               |
| Liquiritin   | 6.06±0.010             | 1.53±0.040 | 0.86±0.010    |
| Glycyrrhizin | 6.92±0.070             | 1.85±0.000 | 0.92±0.012    |
| Naingin      | 6.29±0.050             | 4.52±0.100 | 5.98±0.020    |
| Hesperidin   | 5.68±0.025             | 4.10±0.060 | 5.68±0.025    |
| Ferulic acid | 0.40±0.000             | 0.30±0.000 | 0.33±0.000    |
| Albiflorin   | 0.25±0.000             | 2.14±0.010 | 0.15±0.000    |
| Nodakenin    | 0.30±0.000             | 1.00±0.000 | 0.25±0.000    |
| Cinnamaldehyde | 0.25±0.000     | 0.12±0.000 |               |

The data are presented as the mean±SD from three independent experiments in triplicate.

**Figure 1:** The three-dimensional chromatograms of Bangpungtongseong-san (a), Ojeok-san (b) and oyaksungi-san (c) from high-performance liquid chromatography-photo diode array.
Effects of herbal formulas on the activities of cytochrome P450s

In vitro CYP450 isozyme assays were performed to evaluate whether the three traditional herbal formulas influence the activities of CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4. As shown in Figures 2-4 and Table 4, α-naphthoflavone, sulfaphenazole, quinidine, diethyldithiocarbamate, and ketoconazole were used as positive controls for CYP1A2, CYP2C9, CYP2D6, CYP2E1, and CYP3A4, respectively. Miconazole was used as a positive control for CYP2B6 and CYP2C19. The data are presented as the mean ± standard error of the mean (n = 3).

Figure 2: The effects of Bangpungtongseong-san on the activities of CYP1A2 (a), CYP2B6 (b), CYP2C9 (c), CYP2C19 (d), CYP2D6 (e), CYP2E1 (f), and CYP3A4 (g). The fluorescence-based enzyme assays of the CYP450 isozymes were established in vitro. α-Naphthoflavone, sulfaphenazole, quinidine, sodium diethyldithiocarbamate trihydrate, and ketoconazole were used as positive controls for CYP1A2, CYP2C9, CYP2D6, CYP2E1, and CYP3A4, respectively. Miconazole was used as a positive control for CYP2B6 and CYP2C19. The data are presented as the mean ± standard error of the mean (n = 3).
Figure 3: The effects of Ojok-san on the activities of CYP1A2 (a), CYP2B6 (b), CYP2C9 (c), CYP2C19 (d), CYP2D6 (e), CYP2E1 (f), and CYP3A4 (g). The fluorescence-based enzyme assays of the CYP450 isozymes were established in vitro. α-Naphthoflavone, sulfaphenazole, quinidine, sodium diethyldithiocarbamate trihydrate, and ketoconazole were used as positive controls for CYP1A2, CYP2C9, CYP2D6, CYP2E1, and CYP3A4, respectively. Miconazole was used as a positive control for CYP2B6 and CYP2C19. The data are presented as the mean ± standard error of the mean (n = 3).
Figure 4: The effects of oyaksungi-san on the activities of CYP1A2 (a), CYP2B6 (b), CYP2C9 (c), CYP2C19 (d), CYP2D6 (e), CYP2E1 (f), and CYP3A4 (g). The fluorescence-based enzyme assays of the CYP450 isozymes were established in vitro. α-Naphtho flavone, sulphanilazole, quinidine, sodium diethyldithiocarbamate trihydrate, and ketoconazole were used as positive controls for CYP1A2, CYP2C9, CYP2D6, CYP2E1 and CYP3A4, respectively. Miconazole was used as a positive control for CYP2B6 and CYP2C19. The data are presented as the mean ± standard error of the mean (n = 3).
Effects of Bangpungtongseong-san on the activities of cytochrome P450s

As presented in Figure 2 and Table 4, BPTSS inhibited the activities of CYP1A2, CYP2C19, and CYP2E1, with respective IC$_{50}$ values of 141.77, 94.14, and 104.86 μg/mL. In contrast, BPTSS exerted a relatively weak inhibition on the activities of CYP2B6, CYP2C9, CYP2D6, and CYP3A4, with IC$_{50}$ values ranging from 1.29 to 3.49 μM and 1.56 to 1.93 μM, respectively [Figures 2 and Table 4].

Effects of Ojeok-san on the activities of cytochrome P450s

As shown in Figure 3 and Table 4, OJS exerted the most potent inhibition of the activity of CYP2B6, with an IC$_{50}$ value of 88.92 μg/mL. In addition, OJS inhibited the activity of CYP1A2, with an IC$_{50}$ value of 191.30 μg/mL, whereas it showed competitively weak inhibition on the activities of CYP2C9, CYP2C19, CYP2E1, and CYP3A4, with respective IC$_{50}$ values of 868.74, 252.25, 357.30, and 583.60 μg/mL [Figures 3 and Table 4]. Conversely, OJS inhibited the activity of CYP2B6 in a dose-dependent manner, but the inhibition at 1000 μg/mL OJS did not reach 50%.

Effects of oyaksungi-san on the activities of cytochrome P450s

As demonstrated by the data in Figure 4 and Table 4, OYSGS inhibited the CYP2D6 activity, with an IC$_{50}$ value of 141.47 μg/mL, followed by the activities of CYP1A2 (IC$_{50}$ = 227.11 μg/mL), and CYP2E1 (IC$_{50}$ = 249.84 μg/mL). In addition, OYSGS inhibited the activities of CYP3A4 and CYP2C19, with similar IC$_{50}$ values of 347.00 and 386.41 μg/mL, respectively [Figures 4 and Table 4]. In contrast, OYSGS exhibited the negligible inhibition of both the activities of CYP2B6 and CYP2C9, with IC$_{50}$ values in excess of 1000 μg/mL.

Effects of herbal formulas on the activities of UDP-glucuronosyltransferases

In vitro UGT isozyme assays were performed to investigate the effects of the three traditional herbal formulas on the activities of UGT1A1, UGT1A4, and UGT2B7. As shown in Figures 5-7 and Table 5, diclofenac, which was used as a positive control for UGT1A1 and UGT2B7 in a dose-dependent manner, had IC$_{50}$ values ranging from 295.87 to 823.95 μM and 41.15 to 81.97 μM, respectively. Lopinavir was used as a positive control for UGT1A4, and it was inhibited the CYP1A4 activity in a dose-dependent manner, with an IC$_{50}$ value ranging from 51.88 to 96.41 μM.

Effect of Bangpungtongseong-san on the activities of UDP-glucuronosyltransferases

As shown in Figure 5 and Table 5, BPTSS inhibited the UGT1A1 activity, with an IC$_{50}$ value of 136.36 μg/mL. In contrast, BPTSS inhibited the activities of UGT1A4 and UGT2B7 in a dose-dependent manner, but inhibition at 1000 μg/mL did not reach 50% [Figure 5 and Table 5].

Effect of Ojeok-san on the activities of UDP-glucuronosyltransferases

OJS showed a dose-dependent inhibition on the UGT1A1 activity, but the IC$_{50}$ value was higher than 1000 μg/mL [Figure 6 and Table 5]. In contrast, OJS did not affect the activities of UGT1A4 and UGT2B7 at doses <1000 μg/mL [Figure 6].

Effect of oyaksungi-san on the activities of UDP-glucuronosyltransferases

As presented in Figure 7 and Table 5, OYSGS exhibited a competitively weak inhibition on the UGT1A1 activity, with an IC$_{50}$ value of 824.57 μg/mL. Furthermore, OYSGS inhibited the activity of UGT2B7 in a dose-dependent manner, but inhibition at 1000 μg/mL did not reach 50% [Figure 7]. Conversely, OYSGS showed no inhibition of the UGT1A4 activity at doses <1000 μg/mL [Figure 7].

DISCUSSION

According to the increasing interest in the importance of herb-drug interactions in clinical settings,[25,26] in the study, the effects of traditional herbal formulas (BPTSS, OJS, and OYSGS) that are used to treat MSDs on the activities of CYP450 isozymes (CYP1A2, CYP2C9, CYP2D6, CYP2C19, CYP2D6, and CYP2E1) and UGT isozymes (UGT1A1, UGT1A4, and UGT2B7) were examined. Several reports have demonstrated the influence of herbal extracts or the components present in these herbal formulas on the activities of CYP450s and UGTs. Among them, Glycyrrhizae Radix and Cnidii Rhizoma of BPTSS, OJS, and OYSGS inhibit the activity of human

### Table 4: The IC$_{50}$ values (μg/mL) of the herbal formula extracts on the activities of CYP450 isozymes

| Herbal formula             | CYP1A2 | CYP2B6 | CYP2C9 | CYP2C19 | CYP2D6 | CYP2E1 | CYP3A4 |
|---------------------------|--------|--------|--------|---------|--------|--------|--------|
| Bangpungtongseong-san     | 141.77 | 446.35 | 740.94 | 94.14   | 455.56 | 104.86 | 363.39 |
| Ojeok-san                 | 191.30 | > 1000 | > 1000 | 252.25  | > 1000 | 252.25 | > 1000 |
| Oyaksungi-san             | 227.11 | > 1000 | > 1000 | 386.41  | > 1000 | 141.47 | 249.84 |
| Positive control          | 0.28-0.45 μM | 1.29-3.49 μM | 0.38 μM | 1.56-1.93 μM | 5.23-11.72 nM | 6.47-12.41 μM | 1.33-2.70 nM |

a-Naphthoflavone, sulfaphenazole, quinidine, sodium diethyldithiocarbamate trihydrate and ketoconazole were used as positive controls for CYP1A2, CYP2C9, CYP2D6, CYP2C19 and CYP3A4, respectively. Miconazole was used as a positive control for CYP2B6 and CYP2C19. The values are the means of triplicate experiments.

### Table 5: The IC$_{50}$ values (μg/mL) of the herbal formula extracts on the activities of UGT isozymes

| Herbal formula          | UGT1A1 | UGT1A4 | UGT2B7 |
|------------------------|--------|--------|--------|
| Bangpungtongseong-san  | 136.36 | > 1000 | > 1000 |
| Ojeok-san              | > 1000 | > 1000 | > 1000 |
| Oyaksungi-san          | 824.57 | > 1000 | > 1000 |
| Positive control       | 295.87-823.95 μM | 51.88-96.41 μM | 41.15-81.97 μM |

Diclofenac was used as a positive control for UGT1A1 and UGT2B7. Lopinavir was used as a positive control for UGT1A4. The values are the means of duplicate experiments.
UGT1A1 and rat CYP1A1, respectively. In addition, it has been reported that Scutellariae Radix of BPTSS induces and suppresses the levels of CYP1A and CYP2B in rats, respectively. Scutellariae Radix also inhibits the activity of UGT1A1 in humans. Cinnamomi Bark of OJS inhibits the activities of CYP1A2, CYP2C8, CYP2C9, CYP2D6, and UGT1A1 in humans and CYP1A2 and CYP2C11 in rats. Pseudoephedrine, one of the components in Ephedrae Herba of BPTSS, OJS, and OYSGS, inhibits the activities of CYP1A1/2 and CYP2E1 in rats. Moreover, decursin in Angelicae Gigantis Radix of BPTSS and OJS has inhibitory effects on the activities of CYP1A1/2, CYP3A12, and CYP2D15 in canines. The chromatographic analysis of the various marker components in these herbal formulas was performed using an HPLC-PDA. Among them, geniposide of BPTSS has been reported to decrease the activities of liver microsomal CYP2E1 in mice. Furthermore, baicalin of BPTSS has an inhibitory effect on CYP1A1, CYP2B1, and CYP2C11 in mice. Glycyrrhizin, which is one of the marker compounds of BPTSS and OYSGS, has been reported to inhibit the activities of CYP1A2 in human. In addition, naringin of OJS and OYSGS reduces the CYP1A2 protein level in mice. Cinnamaldehyde of OJS showed inhibits UGT1A1 in humans. However, the major and/or active compounds of herbal formulas are not known. It is difficult to specifically comprehend the metabolizing mechanisms of medicinal herbs, including herbal formulas because they contain a complex group of hundreds of constituent molecules. To date, the effects of herbal formulas, such as BPTSS, OJS, and OYSGS on the activities of CYP450s and UGTs have not been elucidated. In this study, we investigated the inhibitory effects of BPTSS, OJS, and OYSGS on the activities of human CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, UGT1A1, UGT1A4, and UGT2B7 to assess their clinical significance in herb-drug interactions.

The CYP3A family contributes to approximately 50% of the total CYP450 activity in the human liver and is involved in the metabolism of approximately 60% of therapeutic substances. CYP3A4 is the dominant CYP3A enzyme and is expressed in the human liver and gastrointestinal tract. CYP3A4 also has genetic polymorphisms. Some of the most serious CYP-mediated drug interactions are caused by the accumulation of substrates that are metabolized by CYP3A4, such as astemizole, terfenadine, and cisapride. CYP2D6 exhibits genetic polymorphisms and represents <5% of the total CYP proteins. More than 80 drugs in current clinical use are metabolized by CYP2D6, which has aroused great interest because of its large number of substrates (30–50 drugs) and its genetic polymorphism. CYP2C19 also exhibits genetic polymorphisms and has a number of commonly used substrates, including the benzodiazepine diazepam, the proton-pump inhibitor omeprazole, propranolol, and the antidepressive amitriptyline. Few drugs, other than chlorzoxazone and several inhalation anesthetics, are metabolized by CYP2E1. Moreover, CYP2E1 is responsible for the metabolism of ethanol, and chronic ethanol consumption can induce CYP2E1. In this study, BPTSS inhibited the activities of CYP1A2, CYP2C19, CYP2E1, and UGT1A1, which may have clinical implications. Therefore, caution should be exercised when coadministering BPTSS with substrates/inhibitors of CYP1A2, CYP2C19, CYP2E1, or UGT1A1. In addition,
Figure 6: The effects of Ojeok-san on the activities of UGT1A1 (a), UGT1A4 (b), and UGT2B7 (c). The luminescence-based enzyme assays of the UGT isozymes were established in vitro. Diclofenac was used as a positive control for UGT1A1 and UGT2B7. Lopinavir was used as a positive control for UGT1A4. The data are presented as the mean ± standard error of the mean (n = 2).

Figure 7: The effects of oyaksungi-san on the activities of UGT1A1 (a), UGT1A4 (b), and UGT2B7 (c). The luminescence-based enzyme assays of the UGT isozymes were established in vitro. Diclofenac was used as a positive control for UGT1A1 and UGT2B7. Lopinavir was used as a positive control for UGT1A4. The data are presented as the mean ± standard error of the mean (n = 2).
these results suggest that glycyrrhizin and geniposide of BPTSS would contribute to the inhibition of CYP1A2 and CYP2E1, respectively, by BPTSS.\textsuperscript{33,43} In contrast, BPTSS are unlikely to inhibit the metabolism of drugs metabolized by CYP2B6, CYP2C9, CYP2D6, CYP3A4, UGT1A4, and UGT2B7. OJS inhibited the CYP2D6 activity most potently, followed by CYP1A2, CYP2C19, CYP2E1, CYP3A1, and CYP2C9. Thus, attention should be paid when OJS is administered simultaneously with drugs that are metabolized by CYP2D6, and OJS may influence the metabolic reactions mediated by CYP1A2, CYP2C9, CYP2C19, CYP2E1, or CYP3A4 at a high concentration. In contrast, OJS negligibly inhibited the activities of CYP2B6 and UGT1A1, with IC\textsubscript{50} values in excess of 1000 μg/mL, and OJS not influence the activities of UGT1A4 and UGT2B7 at doses <1000 μg/mL. Thus, OJS would not affect CYP2B6, UGT1A1, UGT1A4, or UGT2B7-mediated metabolism in the clinic. OYS showed potent inhibition of the activity of CYP2D6 followed by CYP1A2, CYP2E1, CYP3A4, CYP2C19, and UGT1A1, but it had no significant inhibition on CYP2B6, CYP2C9, UGT1A4, and UGT2B7 at concentrations of over 1000 μg/mL. These findings indicate that OYS is an inhibitor of CYP2D6 and that caution is necessary to reduce its adverse effects when it is coadministered with a substrate/inhibitor of CYP2D6. Furthermore, OYS has a relatively low potential to be involved in herb-drug interactions when administered simultaneously with substrates or inhibitors of CYP2B6, CYP2C9, UGT1A4, or UGT2B7.

In general, aceclofenac and aspirin, which are used for the treatment of rheumatoid arthritis 2 and osteoarthritis, are metabolized by CYP2C9.\textsuperscript{42,43} In addition, naproxen, which is used to treat pain or inflammation caused by arthritis, ankylosing spondylitis, tendinitis, and gout, is a substrate of CYP1A2 and CYP2C9.\textsuperscript{44} and the drug also inhibits the UGT2B7 activity.\textsuperscript{45} Acetaminophen, which is widely used to treat muscle aches, arthritis, backache, and toothache, is metabolized by CYP3A4, CYP2D6, and CYP2E1.\textsuperscript{46,48} Therefore, BPTSS, OJS, and OYS showed potent inhibition of the activity of CYP2D6 followed by CYP1A2, CYP2E1, CYP3A4, CYP2C19, and UGT1A1, but it had no significant inhibition on CYP2B6, CYP2C9, UGT1A4, and UGT2B7 at concentrations of over 1000 μg/mL. These findings indicate that OYS is an inhibitor of CYP2D6 and that caution is necessary to reduce its adverse effects when it is coadministered with a substrate/inhibitor of CYP2D6. Furthermore, OYS has a relatively low potential to be involved in herb-drug interactions when administered simultaneously with substrates or inhibitors of CYP2B6, CYP2C9, UGT1A4, or UGT2B7.

CONCLUSIONS

This study provided information regarding the risks and benefits potentially associated with the use of BPTSS, OJS, and OYS. Caution is necessary when BPTSS is administered together with a substrate/inhibitor of CYP1A2, CYP2C19, CYP2E1, or UGT1A1. Furthermore, herb-drug interactions can occur when OJS or OYS is used in combination with other drugs that are metabolized by CYP2D6, to a greater extent than those that are metabolized by other isozymes.

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Conflicts of interest

There are no conflicts of interest.

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