Inhibition of human liver microsomal CYP3A4 and CYP2D6 by extracts from 78 herbal medicines

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The inhibitory effects of 78 herbal extracts on cytochrome P450 3A4 (CYP3A4) and P450 2D6 (CYP2D6) activity were investigated using human liver microsomes. The incubation mixture contained a methanol soluble fraction prepared from the powder of each herbal water extract (equivalent to 1.65 mg of extract powder per mL). Thirty-one herbal extracts inhibited over 50% of human liver microsomal erythromycin *N*-demethylation, a marker reaction of CYP3A4 activity. Among the 31 herbal extracts, 8 of them (Angelica Dahurica Root, Cassia Bark, Clove, Incised Notopterygium Rhizome, Moutan Bark, Rhubarb, Sappan Wood, Schisandra Fruit) inhibited *N*-demethylation by over 90%. Among the herbal extracts examined, the strongest inhibition of CYP3A4 was noted with Sappan Wood, which had an IC_{50} value of 43 mg/mL. Rhubarb, Schisandra Fruit, Incised Notopterygium Rhizome, and Angelica Dahurica Root had IC_{50} values of 77, 127, 144, and 185 μg/mL, respectively. Further, 28 of the herbal extracts inhibited over 50% of human liver microsomal dextromethorphan *O*-demethylation, which is a marker of CYP2D6 activity. Among the 28 herbal extracts, 13 (Cassia Bark, Clove, Coptis Rhizome, Ephedra Herb, Gambir Plant, Incised Notopterygium Rhizome, Magnolia Bark, Moutan Bark, Phellodendron Bark, Rhubarb, Sappan Wood, Sinomenium Stem, Zanthoxylum Fruit) inhibited *O*-demethylation by over 90%. The strongest inhibition of CYP2D6 was noted with Phellodendron Bark, which had an IC_{50} value of 4 μg/mL. Coptis Rhizome, Sinomenium Stem, Sappan Wood, and Rhubarb showed IC_{50} values of 14, 40, 52, and 64 μg/mL, respectively. These results indicate that many herbal extracts have an inhibitory effect on CYP3A4 and CYP2D6.

Key words herb, Kampo medicines, human liver microsomes, inhibition, CYP3A4, CYP2D6.

Abbreviations CYP, Cytochrome P450; G-6-P, glucose 6-phosphate; NADPH, nicotinamide adenine dinucleotide phosphate reduced form; $NADP^+$, nicotinamide adenine dinucleotide phosphate oxidized form.

Introduction

Coadministration of multiple drugs is known to cause many drug－drug interactions in clinical situations. The largest cause of interaction is pharmacokinetic factors such as inhibition or induction of cytochrome P450 (CYP). CYP superfamillies are composed of families; four of the families have been identified as having the ability to catalyze the oxidation of many drugs. CYP3A4, the major hepatic and intestinal CYP isoform in human, metabolizes more than 50% of all clinically used drugs. CYP2D6 is also an important isoform, metabolizing about 30% of all clinically used drugs.[1] It is therefore important to consider effects on CYP in order to predict drug interactions.[2,3]

The global demand for traditional medicines is increasing year by year. In recent years, the safety and efficacy of herbs have been scientifically evaluated.[4] In Japan, herbal medicines are known as Kampo medicines and 210 prescriptions consisting of 5－10 different herbs have been approved by the Ministry of Heath and Welfare (MHW). One hundred and forty-six different herbs are used in the 210 prescriptions. Herbal medicines are defined as medicines in many European countries, while they are classified as dietary supplement in the United States.[5] According to a questionnaire on Kampo medicines conducted in Japan in 2000, more than 70% of clinical physicians were prescribing Kampo medicines to their patients.[6]

These results suggest that herbal medicines are often used with concomitantly with synthetic drugs. Clinical case reports of herb-drug interactions involving top-selling herbs such as Ginkgo (*Ginkgo biloba*), St. John's wort (*Hypericum perforatum*), Ginseng (*Panax ginseng*), Garlic (*Allium sativum*), Kava (*Piper methysticum*) have been reported in U.S.[7,8] Coadministration of St. John's wort lowers the blood concentrations of cyclosporine,[9] warfarin,[10] digoxin,[11] and many other drugs which are metabolized by CYP3A4. Moreover, St. John's wort has been shown to induce intestinal P-glycoprotein and hepatic CYP3A4.[12] However, CYP inhibition plays a more important role in the mechanism of drug interaction than in CYP induction. As a typical example, coadministration of grapefruit juice is well known to increase the bioavailabilities of many clinically important drugs such as calcium channel blockers,[13-15] cyclosporin,[16] midazolam,[17] and terfenadine,[18] through a strong inhibition of CYP3A4 by furanocoumarin derivatives contained in

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grapefruit juice.[19] To avoid interactions between herbs and drugs, it is necessary to clearly define the inhibitory potential of different herbs. However, no systematic investigation has been conducted on the inhibition of CYPs by herbs used in Kampo medicines.

In the present study, 78 herbal extracts used in Kampo medicines were tested for their inhibitory effects on human liver microsomal CYP3A4 and CYP2D6 activities. Our results indicate that many herbal extracts inhibit CYP3A4 and CYP2D6 activities in a CYP-selective and extract-specific manner.

Materials and Methods

Materials. Powders of the 78 herbal extracts listed in Table 1 were kindly provided by Tsumura Ltd. (Tokyo, Japan). Each herbal extract powder was prepared by immersing the herb in distilled water and boiling it at 95℃, then passing it through a filter. The filtrate was evaporated under reduced pressure and then spray-dried to give the powder. [*N*-methyl-^{14}C]Erythromycin (2.035 GBq/mmol; radiochemical purity >99%) and [*O*-methyl-^{14}C] dextromethorphan (2.035 GBq/mmol; radiochemical purity > 99%) were purchased from American Radiochemicals Inc. (St. Louis, MO, USA). Erythromycin, dextromethorphan hydrobromide monohydrate, ketoconazole, and quinidine were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Nicotinamide adenine dinucleotide phosphate oxidized form ($NADP^+$), glucose 6-phosphate (G-6-P), and G-6-P dehydrogenase were purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). All other reagents and solvents were analytical grade and were supplied by Wako Pure Chemical Industries, Ltd.

Human liver microsomes. Human liver microsomes (HLM), which were prepared from 16 individuals (male, 11; female, 5), were obtained from Xeno Tech LLC (Kansas City, KS, USA) and stored at -80℃ until use. The content of CYP in the microsomes was 0.385 nmol/mg protein and the specific activity of each CYP isoform was as follows: CYP1A2 (7-ethoxyresorufin *O*-deethylation), 35.4 ± 2.4 pmol/mg protein/min; CYP2A6 (coumarin 7-hydroxylation), 921 ± 22 pmol/mg protein/min; CYP2B6 (*S*-mephenytoin

Table 1. List of the herbs used in this study

No.	English name	Latine name	Japanese name		Family name	Lot No.
1	Achyranthes Root	Achyranthis Radix	牛膝	Goshitsu	Amaranthaceae	251066010
2	Akebia Stem	Akebiae Caulis	木通	Mokutu	Lardizabalaceae	241106010
3	Alisma Rhizome	Alismatis Rhizoma	沢瀉	Takusha	Alismataceae	241069010
4	Amomum Seed	Amomi Semen	縮砂	Syukusha	Zingiberaceae	271103010
5	Anemarrhena Rhizome	Anemarrhenae Rhizoma	知母	Chimo	Liliaceae	281071010
6	Angelica Dahurica Root	Angelicae Dahuricae Radix	白芷	Byakushi	Umbelliferae	231076010
7	Apricot Kernel	Armeniacae Semen	杏仁	Tonin	Rosaceae	251046010
8	Argy Wormwood Leaf	Artemisiae Folium	艾葉	Gaiyo	Compositae	251087010
9	Artemisiae Capillaris Spica	Artemisiae Capillari Flos	茵陳蒿	Inchinko	Compositae	2001085010
10	Asiasarum Root	Asiasari Radix	細辛	Saishin	Aristolochiaceae	2001026010
11	Astragalus Root	Astragali Radix	黄耆	Ogi	Leguminosae	2001023010
12	Atractylodes Lancea Rhizome	Atractylodis Lanceae Rhizoma	蒼朮	Sojutsu	Compositae	281005010
13	Atractylodes Rhizome	Atractylodis Rhizoma	白朮	Byakujutsu	Compositae	2991077010
14	Bupleurum Root	Bupleuri Radix	柴胡	Saiko	Umbelliferae	281020010
15	Cassia Bark	Cinnamomi Cortex	桂皮	Keihi	Lauraceae	271003020
16	Chinese Wolfberry Root-bark	Lycii Cortex	地骨皮	Jikkopi	Solanaceae	241092010
17	Chrysanthemum	Chrysanthemi Flos	菊花	Kikka	Compositae	2991110010
18	Chuling	Polyporus	猪苓	Chorei	Polyporaceae	2991099010
19	Cimicifuga Rhizome	Cimicifugae Rhizoma	升麻	Shoma	Ranunculaceae	281081010
20	Citrus Unshu Peel	Aurantii Nobilis Pericarpium	陳皮	Chinpi	Rutaceae	241009010
21	Clove	Caryophylli Flos	丁子	Choji	Myrtaceae	2991017010
22	Cnidium Rhizome	Cnidii Rhizoma	川芎	Senkyu	Umbelliferae	281004010
23	Coix Seed	Coicis Semen	薏苡仁	Yokuinin	Gramineae	251052020
24	Coptis Rhizome	Coptidis Rhizoma	黄連	Ouren	Ranunculaceae	2001014010
25	Cornus Fruit	Corni Fructus	山茱萸	Sanshuyu	Cornaceae	281095010
26	Corydalis Tuber	Corydalis Tuber	延胡索	Engosaku	Papaveraceae	251019010
27	Dioscorea Rhizome	Dioscoreae Rhizoma	山薬	Sanyaku	Dioscoreaceae	281027010
28	Ephedra Herb	Ephedrae Herba	麻黄	Mao	Ephedraceae	2991037010
29	Evodia Fruit	Evodiae Fructus	呉茱萸	Gosyuyu	Rutaceae	2991042010

Tabble 1. (continued)

30	Forsythia Fruit	Forsythiae Fructus	連翹	Rengyo	Oleaceae	271097010
31	Gambir Plant	Uncariae Uncis Cum Ramulus	釣藤鉤	Chotoko	Rubiaceae	291089010
32	Gardenia Fruit	Gardeniae Fructus	山梔子	Sanshishi	Rubiaceae	2001044010
33	Ginger	Zingiberis Rhizoma	生姜	Shokyou	Zingiberaceae	281016010
34	Ginger	Zingiberis Siccatum Rhizoma	乾姜	Kankyou	Zingiberaceae	2001067010
35	Ginseng	Ginseng Radix	人参	Ninjin	Araliaceae	271015010
36	Glehnia Root	Glehniae Radix Cum Rhizoma	浜防風	Hamabofu	Umbelliferae	291022010
37	Great Burdock Achene	Arctii Fructus	牛蒡子	Goboshi	Compositae	281047010
38	Immature Orange	Aurantii Fructus Immaturus	枳実	Kijitsu	Rutaceae	241041010
39	Incised Notopterygium Rhizome	Notopterygii Rhizoma	羌活	Kyokatsu	Umbelliferae	251136010
40	Japanese Angelica Root	Angelicae Radix	当帰	Toki	Umbelliferae	261002010
41	Japanese Gentian	Gentianae Scabrae Radix	竜胆	Ryutan	Gentianaceae	281080010
42	Jujube	Zizyphi Fructus	大棗	Taiso	Rhamnaceae	2991039011
43	Lancelesf Lily Bulb	Lilii Bulbus	百合	Byakugo	Liliaceae	271141010
44	Licorice Root	Glycyrrhizae Radix	甘草	Kanzo	Leguminosae	281013010
45	Licorice Root	Glycyrrhizae Radix	炙甘草	Shakanzo	Leguminosae	251122010
46	Loquat Leaf	Eriobotryae Folium	枇杷葉	Biwayo	Rosaceae	271142010
47	Magnolia Bark	Magnoliae Cortex	厚朴	Koboku	Magnoliaceae	241035010
48	Mentha Herb	Menthae Herba	薄荷	Hakka	Labiatae	2991036010
49	Moutan Bark	Moutan Cortex	牡丹皮	Botampi	Paeoniaceae	251006010
50	Nelumbo nucifera Gaertn	Nelumbis Semen	蓮肉	Renniku	Nymphaeaceae	241124010
51	Ophiopogon Tuber	Ophiopogonis Tuber	麦門冬	Bakumondo	Liliaceae	261075010
52	Paeony Root	Paeoniae Radix	芍薬	Syakuyaku	Paeoniaceae	281001010
53	Peach Kernel	Persicae Semen	桃仁	Tonin	Rosaceae	241012010
54	Perilla Herb	Perillae Herba	蘇葉	Soyo	Labiatae	271091010
55	Phellodendron Bark	Phellodendri Cortex	黄柏	Obaku	Rutaceae	2001034010
56	Pinellia Tuber	Pinelliae Tuber	半夏	Hange	Araceae	281032010
57	Plantago Seed	Plantaginis Semen	車前子	Shazenshi	Plantaginaceae	281049010
58	Platycodon Root	Platycodi Radix	桔梗	Kikyou	Campanulaceae	251025010
59	Polygala Root	Polygalae Radix	遠志	Onji	Polygalaceae	281033010
60	Poria Sclerotium	Poria	茯苓	Bukuryo	Polyporaceae	271007020
61	Pueraria Root	Puerariae Radix	葛根	Kakkon	Leguminosae	251021010
62	Rehmannia Root	Rehmanniae Radix	地黄	Jio	Scrophulariaceae	261011010
63	Rhubarb	Rhei Rhizoma	大黄	Daio	Polygonaceae	2991028010
64	Rice	Oryzae Semen	粳米	Kobei	Gramineae	241104010
65	Safflower	Carthami Flos	紅花	Koka	Compositae	251126020
66	Saina Data Seed	Zizyphi Spinishi Semen	酸棗仁	Sansonin	Rhamnaceae	281048010
67	Saposhnikoviae Root	Saposhnikoviae Radix	防風	Bofu	Umbelliferae	2991031010
68	Sappan Wood	Sappan Lignum	蘇木	Soboku	Leguminosae	271134010
69	Saussurea Root	Saussureae Radix	木香	Mokko	Compositae	251079010
70	Schisandra Fruit	Schisandrae Fructus	五味子	Gomishi	Schisandraceae	291043010
71	Schizonepeta Spike	Schizonepetae Spica	荊芥	Keigai	Labiatae	2991038010
72	Scutellaria Root	Scutellariae Radix	黄	Ogon	Labiatae	281024010
73	Sinomenium Stem	Sinomeni Caulis et Rhizoma	防已	Boi	Menispermaceae	261029010
74	Sophora Flower	Magnoliae Flos	辛夷	Shini	Magnoliaceae	2991054010
75	Sophora Root	Sophorae Radix	苦参	Kujin	Leguminosae	281065010
76	Trichosanthes Root	Trichosanthis Radix	栝楼根	Karokon	Cucurbitaceae	2011063010
77	Whiteflower Hogfennel Root	Peucedani Radix	前胡	Zenko	Umbelliferae	2011068010
78	Zanthoxylum Fruit	Zanthoxyli Fructus	山椒	Sansho	Rutaceae	251094010

Herbs are listed in the alphabetical order of their English names.

N-demethylation), 57.7 ± 1.4 pmol/mg protein/min; CYP2C8 (taxol 6α-hydroxylation), 546 ± 26 pmol/mg protein/min; CYP2C9 (diclofenac 4'-hydroxylation), 1300 ± 60 pmol/mg protein/min; CYP2C19 (*S*-mephenytoin 4'-hydroxylation), 108 ± 2 pmol/mg protein/min; CYP2D6 (dextromethorphan *O*-demethylation), 228 ± 4 pmol/mg protein/min; CYP2E1 (chlorzoxazone 6-hydroxylation), 1780 ± 10 pmol/mg protein/min; CYP3A4/5 (testosterone 6β-hydroxylation), 3100 ± 30 pmol/mg protein/min; CYP4A9/11 (lauric acid 12-hydroxylation), 1370 ± 22.8 pmol/mg protein/min.

Preparation of methanol and diethyl ether soluble fractions from water extracts of herbs. The powder of each herbal extract (1 g) was mixed with 20 mL of methanol and the mixture was vigorously shaken for 1 hr at room temperature. The mixture was centrifuged at 1600*g* for 10 min, and then the methanol layer was separated. The precipitate was extracted again with 10 mL of methanol in the same manner. Both methanol solution (30 mL) was combined, and 5 mL of the solution was evaporated to dryness under a nitrogen stream. The residue was dissolved in 500 μL of methanol and used as the methanol soluble fraction in the inhibition study (Fig. 1). Five milliliters of the methanol solution was evaporated to dryness under a nitrogen stream. The residue was added with 1 mL of water and 3 mL of diethyl ether, and the mixture was vigorously shaken for 10 min. The mixture was centrifuged at 1600*g* for 10 min, and then the diethyl ether layer was separated. This procedure was repeated 2 times. The diethyl ether layer was evaporated to dryness under a nitrogen stream. The residue was dissolved in 500 μL of methanol and used as the diethyl ether soluble fraction in the inhibition study. One microliter of the both methanol and diethyl ether soluble fractions was 0.33 mg equivalent to the powder of the herbal extract.

Enzyme assay. Erythromycin *N*-demethylation and dextromethorphan *O*-demethylation activities in human liver microsomes were used to determine CYP3A4 and CYP2D6 activity, respectively. [*N*-methyl-^{14}C] Erythromycin and [*O*-methyl-^{14}C] dextromethorphan, used as the substrates, were dissolved in methanol. The activity was calculated from the radioactivity of [^{14}C] formaldehyde produced enzymatically.[20,21] The final volume of the mixture was 0.5 mL, and the final concentrations were 0.1 M phosphate buffer (pH 7.4), 0.1 mM EDTA, 0.4 mg/mL microsomal protein, 0.5 mM^{+}, 5 mM G-6-P, 5 mM $MgCl_2$, 1 unit/mL G-6-P dehydrogenase, and 100 μM erythromycin or 10 mM dextromethorphan. The concentration of methanol was 1.0% (v/v) in the reaction mixture. Each incubation mixture containing all the components, except the NADPH-generating system, was preincubated at 37°C for 5 min. Then, the NADPH-generating system was added to the mixture to initiate the metabolic reaction. The mixture was incubated at 37°C for 10 min for erythromycin *N*-demethylation and 20 min for dextromethorphan *O*-demethylation.

After incubation, reactions were stopped by the addition of 10% (v/v) trichloroacetic acid (125 μL), and centrifuged at 1600*g* for 10 min at room temperature. The supernatant was then applied to a preconditioned (1 mL of methanol, 1 mL of water) Envi-Carb solid-phase extraction (SPE) column (100 mg bed volume, Supelco, UK). Samples were eluted with 2 mL of water, and the eluate was mixed with 10 mL of Cleasol I liquid scintillation cocktail (Nacalai Tesque, Kyoto, Japan). Then, the radioactivity was counted for 5 min using a Tri-Carb model 2300TR liquid scintillation counter (Perkin Elmer Life Sciences Japan Co., Ltd., Tokyo, Japan). All incubations were performed in duplicate.

Inhibition of microsomal CYPs with methanol and diethyl ether soluble fractions from herbal extracts. Each methanol soluble or ether soluble fraction of the herbal extract was added to the reaction mixture at an amount equivalent to 1.65 mg of herbal extract powder per mL. To calculate the IC_{50} value of the herbal extract, each methanol soluble fraction was added to the reaction mixture at a concentration range of 0 − 1.65 mg/mL. The relationship between the concentration of herbal extract and the remaining activity of CYP2D6 and CYP3A4 was analyzed using WinNonlin v3.1 pharmacokinetic analysis software (Pharsight Corp., Mountain View, CA, USA). The herbal extract concentration resulting in 50% inhibition of metabolic activity (IC_{50}) was determined. The IC_{50} values for ketoconazole and quinidine were also calculated (0 − 10 μM).

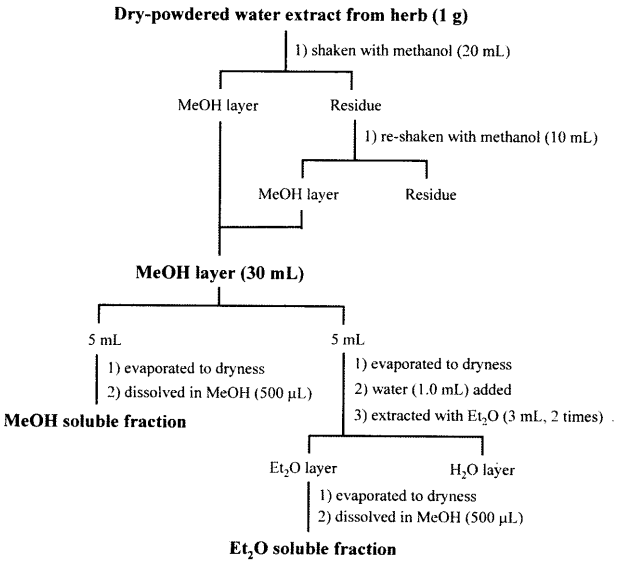

Fig. 1 Fractionation of CYP-inhibitory components from herbal extracts.

Results

Activity and inhibition of human liver microsomal CYP3A4 and CYP2D6 activity determined with probe substrates and inhibitors. Human liver microsomal erythromycin *N*-demethylation activity was 327 pmol/mg protein/min. Ketoconazole, a specific inhibitor of CYP3A4, inhibited the erythromycin *N*-demethylation activity of human liver microsomes in a concentration-dependent manner (IC_{50} = 0.245 μM; Fig. 2A). Moreover, ketoconazole almost completely inhibited erythromycin *N*-demethylation at 10 μM (98% inhibition). Human liver microsomal dextromethorphan *O*-demethylation activity was 161 pmol/mg

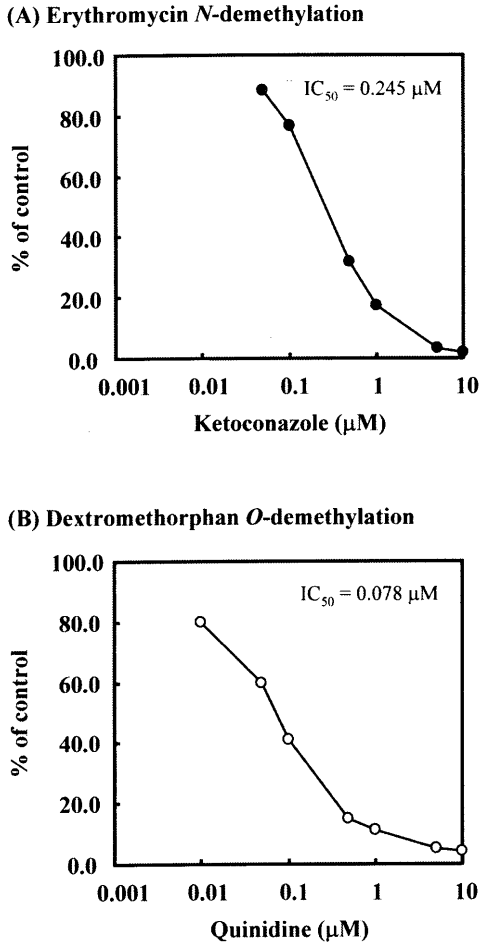

Fig. 2 Inhibitory effects of ketoconazole (A) and quinidine (B) on CYP-specific oxidation catalyzed by human hepatic microsomes. The concentration of microsomal protein was 0.4 mg/mL and the concentration of erythromycin and dextromethorphan used as the substrate were 100 and 10 μM, respectively. Microsomes were incubated for 10 min (erythromycin) or 20 min (dextromethorphan) at 37°C and pH 7.4 in the presence of an NADPH-generating system and 0.1 mM EDTA. Data are expressed as means of duplicate experiments.

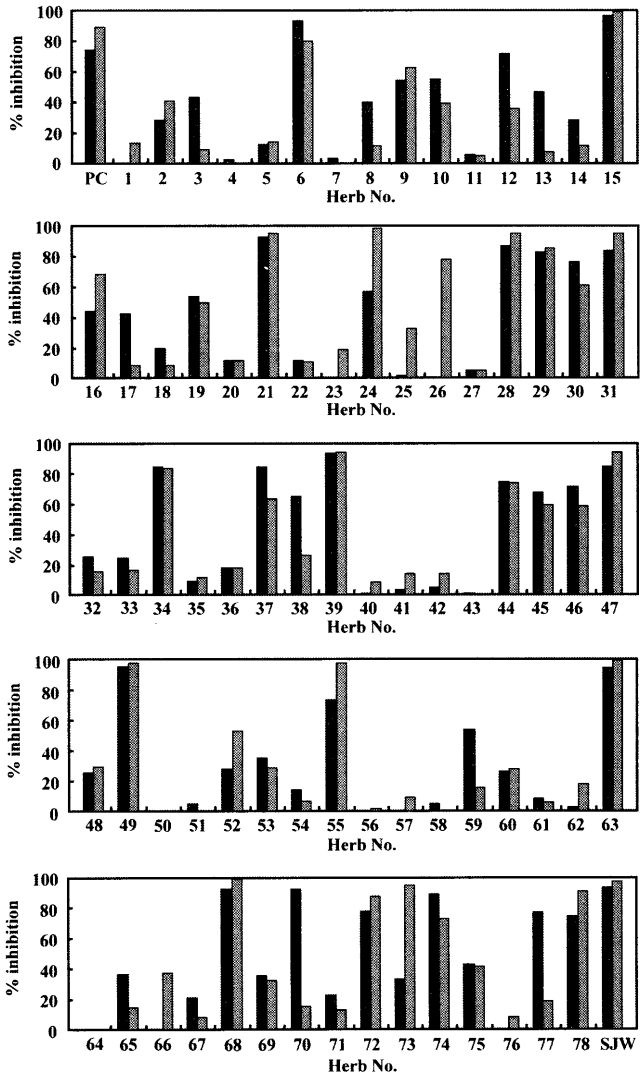

Fig. 3 Inhibition of human liver microsomal CYP3A4 and CYP2D6 activities by methanol soluble fractions from 78 herbal extracts. In the presence of a methanol soluble fraction equivalent to 1.67 mg of each dried herbal extract/mL, microsomal enzyme activities were determined with the substrate, erythromycin (closed bar) for CYP3A4 or dextromethorphan (shaded bar) for CYP2D6, under the same conditions as described in Fig. 2. PC represents a positive control in which, 1 μM ketoconazole for CYP3A4 and 1 μM quinidine for CYP2D6, and SJW, St. John's wort, were used as the inhibitor. Data are expressed as means of duplicate experiments.

protein/min. Quinidine, a specific inhibitor of CYP2D6, inhibited the dextromethorphan *O*-demethylation activity of human liver microsomes in a concentration-dependent manner (IC_{50} = 0.078 μM; Fig. 2B). Furthermore, quinidine almost completely inhibited dextromethorphan *O*-demethylation at 10 μM (95% inhibition).

Inhibitory effects of herbal methanol fractions on CYP3A4 and CYP2D6 activities. Inhibitory effects of 78 herbal methanol fractions on human liver microsomal erythromycin *N*-demethylation and dextromethorphan *O*-demethylation are shown in Fig. 3. Greater than 70% inhibition of erythromycin *N*-demethylation activity was observed with 23 of the herbal methanol fractions: Angelica Dahurica Root, Atractylodes Lancea Rhizome, Cassia Bark, Clove, Ephedra Herb, Evodia Fruit, Forsythia Fruit, Gambir Plant, Ginger, Great Burdock Achene, Incised Notopterygium Rhizome, Licorice Root (Kanzo), Loquat Leaf, Magnolia Bark, Moutan Bark, Phellodendron Bark, Rhubarb, Sappan Wood, Schisandra Fruit, Scutellaria Root, Sophora Flower, Whiteflower Hogfennel Root, and Zanthoxylum Fruit. Partial inhibition (30－70%) of *N*-demethylation was observed with 17 of the herbal methanol fractions: Alisma Rhizome, Argy Wormwood Leaf, Artemisiae Capillaris Spica, Asiasarum Root, Atractylodes Rhizome, Chinese Wolfberry Root-bark, Chrysanthemum, Cimicifuga Rhizome, Coptis Rhizome, Immature Orange, Licorice Root (Shakanzo), Peach Kernel, Polygala Root, Safflower, Saussurea Root, Sinomenium Stem, and Sophora Root. The remaining 38 herbal methanol fractions resulted in less than 30% inhibition of *N*-demethylation.

Greater than 70% inhibition of dextromethorphan *O*-demethylation activity was observed with 20 of the herbal

methanol fractions: Angelica Dahurica Root, Cassia Bark, Clove, Coptis Rhizome, Corydalis Tuber, Ephedra Herb, Evodia Fruit, Gambir Plant, Ginger, Incised Notopterygium Rhizome, Licorice Root (Kanzo), Magnolia Bark, Moutan Bark, Phellodendron Bark, Rhubarb, Sappan Wood, Scutellaria Root, Sinomenium Stem, Sophora Flower, and Zanthoxylum Fruit. Partial inhibition (30 − 70%) of *O*-demethylation was observed with 15 of the herbal methanol fractions: Akebia Stem, Artemisiae Capillaris Spica, Asiasarum Root, Atractylodes Lancea Rhizome, Chinese Wolfberry Root-bark, Cimicifuga Rhizome, Cornus Fruit, Forsythia Fruit, Great Burdock Achene, Licorice Root (Shakanzo), Loquat Leaf, Paeony Root, Saina Data Seed, Saussurea Root, and Sophora Root. The remaining 43 herbal methanol fractions inhibited *O*-demethylation by less than 30%.

Table 2. IC_{50} values of inhibition of methanolic fraction from herbal extracts for human liver microsomal CYP3A4 and CYP2D6 activities

No.[a]	Herbs with English name (Japanese name)	IC_{50} value (μg/mL) [b]	
		CYP3A4	CYP2A6
6	Angelica Dahurica Root	185	405
12	Atractylodes Lanccea Rhizome	474	NA
15	Cassia Bark	226	260
21	Clove	231	230
24	Coptis Rhizome	NA	14
26	Corydalis Tuber	NA	452
28	Ephedra Herb	483	235
29	Evodia Fruit	293	306
30	Forsythia fruit	559	NA
31	Gambir Plant	346	298
34	Ginger (Kankyou)	328	345
37	Great Burdock Achene	247	NA
39	Incised Notopterygium Rhizome	144	120
44	Licorice Root (Kanzo)	628	735
78	Loquat Leaf	724	NA
47	Magnolia Bark	638	183
49	Moutan Bark	311	317
55	Phellodendron Bark	631	4
63	Rhubarb	77	64
68	Sappan Wood	43	52
70	Schisandra Fruit	127	NA
72	Scutellaria Root	496	429
73	Sinomenium Stem	NA	40
74	Sophora Flower	435	634
77	Whiteflower Hogfennel Root	654	NA
78	Zanthoxylum Fruit	671	213
	St. John's wort	72	178

[a]Numbers and names of herbs are the same as listed in Table 1. St. John's wort on the bottom of the table was used as a positive control.
[b]IC_{50} was determined in the same manners as shown in Fig. 2. Erythromycin and dextromethorphan were used as substrates for CYP3A4 and CYP2D6 activities of human liver microsomes.

CYP-inhibitory potencies (IC_{50} values) of herbal methanol fractions. The IC_{50} values for the methanol soluble fractions of herbal extracts that inhibited CYP by more than 70% are listed in Table II. All of the 23 herbal methanol fractions inhibited the activity of erythromycin *N*-demethylation in a concentration-dependent manner. The order of inhibitory potencies and IC_{50} values, from most to least potent, was: Sappan Wood (43 μg/mL), Rhubarb (77 μg/mL), Schisandra Fruit (127 μg/mL), Incised Notopterygium Rhizome (144 μg/mL), and Angelica Dahurica Root (185 μg/mL).

On the other hand, all of the 20 herbal methanol fractions inhibited the activity of dextromethorphan *O*-demethylation in a concentration-dependent manner. The order of inhibitory potencies and IC_{50} values, from most to least potent, was: Phellodendron Bark (4 μg/mL), Coptis Rhizome (14 μg/mL), Sinomenium Stem (40 μg/mL), Sappan Wood (52 μg/mL), and Rhubarb (64 μg/mL).

Diethyl ether extraction of CYP inhibitors from herbal extracts. The inhibitory potential of diethyl ether soluble fractions of each of the herbs on erythromycin *N*-demethylation activity was compared with that of the methanol fractions (Fig. 4), when the methanol soluble fractions resulted in greater than 70% inhibition. The degree of inhibition of erythromycin *N*-demethylation activity was identical between the diethyl ether soluble- and methanol soluble-fractions of Angelica Dahurica Root, Atractylodes Lancea Rhizome, Ginger, Incised Notopterygium Rhizome, Licorice Root (Kanzo), Sappan Wood, Schisandra Fruit, Scutellaria Root, Sophora Flower, Whiteflower Hogfennel Root, and several of the other herbs. The degree of inhibition of dextromethorphan *O*-demethylation activity was identical between the diethyl ether soluble and methanol soluble fractions of Angelica Dahurica Root, Corydalis Tuber, Ginger, Incised Notopterygium Rhizome, Licorice Root (Kanzo), Sappan Wood, Scutellaria Root, Sinomenium Stem, and several of the other herbs.

Discussion

In the present study, we used human liver microsomal erythromycin *N*-demethylation and dextromethorphan *O*-demethylation activities to determine the catalytic activities of CYP3A4 and CYP2D6, respectively. It is known that human liver microsomal erythromycin *N*-demethylation activity was highly correlated with the rate of testosterone 6β-hydroxylation which was a typical marker of CYP3A4 catalytic activity *in vitro* ($r^2 = 0.92$, $p<0.001$, $N = 9$).[20] The *O*-demethylation activity of dextromethorphan is catalyzed by CYP2D6, and this activity has been used as a marker of CYP2D6 catalytic activity both *in vitro*[22,23] and *in vivo*.[24-26] The erythromycin *N*-demethylation activity of human liver microsomes, which was examined in this study, is reported to be inhibited almost completely by ketoconazole,[27] a CYP3A4-specific inhibitor, while dextromethorphan *O*-demethylation activity is inhibited almost completely by quinidine,[27,28] a CYP2D6-specific inhibitor. Therefore, it was confirmed that the human liver microsomes used in this

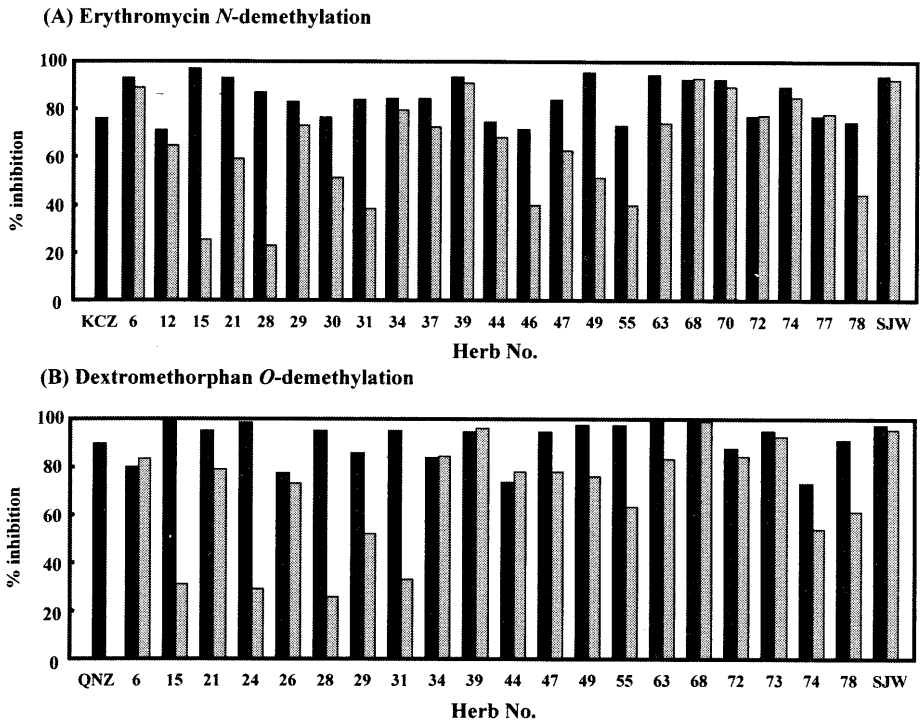

Fig. 4 Inhibition of human liver microsomal CYP3A4 and CYP2D6 activities by methanol and diethyl ether soluble fractions. The methanol soluble fractions (closed bar) and diethyl ether soluble fractions (shaded bar) were extracted from herbals used as illustrated in Fig. 1. The methanol and diethyl ether soluble fractions equivalent to 1.65 mg of each dried herbal extract/mL were incubated with human liver microsomes, under the same conditions as described in Fig. 2. KCZ, ketoconazole (1 μM); QNZ, quinidine (1 μM); and SJW, St. John's wort. Data are expressed as means of duplicate experiments.

study were of sufficient quality to evaluate the CYP3A4 and CYP2D6 activities. [*N*-methyl-^{14}C]Erythromycin and [*O*-methyl-^{14}C]dextromethorphan were used as the substrates for CYP3A4 and CYP2D6, respectively. ^{14}C-Labeled formaldehyde produced from the substrate could be distinguished from that derived from herbal components. In order to reconstruct the reaction conditions *in vivo*, we used human liver microsomes as a source of enzyme and an NADPH-generating system as a source of NADPH in this study. Thus, direct inhibition of NADPH cytochrome P450 reductase or G-6-P dehydrogenase in the NADPH-generating system could be detected as an apparent inhibition of the CYP3A4 and CYP2D6 activities.

In the comparison of IC_{50} values, Sappan Wood showed the strongest inhibition of CYP3A4-mediated activity among all methanol soluble fractions of the herbals used, followed in order by Rhubarb, Schisandra Fruit, Incised Notopterygium Rhizome, and Angelica Dahurica Root. The IC_{50} values of the extracts of Rhubarb, Schisandra Fruit, Incised Notopterygium Rhizome, and Angelica Dahurica Root were 43, 77, 127, 144, and 185 μg/mL, respectively. Guo *et al.* reported that Incised Notopterygium Rhizome and Angelica Dahurica Root contain the powerful inhibitors GF-I-1 and GF-I-4, which are CYP3A4-inhibitory components of grapefruit juice.[29] Ishihara *et al.* reported that in rats coadministered diazepam, Angelica Dahurica Root increased the mean peak plasma concentration (C_{max}) of diazepam about 4-fold, through a marked change in first-pass metabolism.[30] Our results show that the inhibitory effects of Sappan Wood, Rhubarb, and Schisandra Fruit on CYP3A4 activity were stronger than those of Incised Notopterygium Rhizome and Angelica Dahurica Root, which contain GF-I-1 and GF-I-4. Therefore, Sappan Wood, Rhubarb, and Schisandra Fruit should contain powerful inhibitor(s) of CYP3A4. CYP3A4 is the major CYP-isoform expressed in human liver (about 30% of total CYP), and is also the major form in the small intestine.[31,32] Since all of the herbal medicines were orally administered in the present study, herbs containing such powerful inhibitory component(s) could inhibit CYP3A4 in the small intestine.

Phellodendron Bark showed the strongest inhibition of CYP2D6 activity among the methanol soluble herbal fractions tested, followed by Coptis Rhizome and Sinomenium Stem. The IC_{50} values of the extracts of Phellodendron Bark, Coptis Rhizome, and Sinomenium Stem were 4, 14, and 40 μg/mL, respectively. These results suggest that they contain a powerful CYP2D6-inhibitor(s). It is known that substrates for CYP2D6 possess a basic nitrogen atom at either 5－7 Å from the oxidation site and that the aromatic rings are coplanar.[33,34] Berberine is the major alkaloid in Phellodendron Bark and Coptis Rhizome,[35] but the IC_{50} value of berberine to CYP2D6 was over 10 μM (34.8% inhibition at 10 μM). Sinomenine is the major alkaloid in Sinomenium Stem,[36] but its inhibitory effect on CYP2D6 was weak (no effect at 10 μM). These results suggest that inhibitory components against CYP2D6 in Phellodendron Bark, Coptis Rhizome, and Sinomenium Stem are alkaloids other than berberine and sinomenine.

Hasegawa *et al.* identified o-methoxycinnamaldehyde (OMCA) in Cassia Bark as an inhibitor of CYP1A2 and CYP2E1.[37] The inhibitory constants (*Ki* values) for CYP1A2 and CYP2E1 were 16.0 and 7.7 μM, respectively. OMCA also weakly inhibited CYP3A, CYP2C, and CYP2D6. In our study, the inhibition of CYP3A4 and CYP2D6 activity by Cassia Bark extract was relatively weak (IC_{50} values for CYP3A4 and CYP2D6, 226 and 260 μg/mL, respectively). Henderson *et al.* examined the inhibitory effects of 7 ginsenoside and 2 eleutheroside components of Ginseng on 5 CYP isoforms (CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4).[38] Even though the IC_{50} value of the most powerful CYP3A4 inhibitor, ginsenoside Rd, was 58 μM, the authors suggested that ginsenosides and eleutherosides were not likely to inhibit CYP-mediated metabolism of coadministered drugs. In our study, the inhibitory effects of Ginseng methanol soluble fractions on CYP3A4 and CYP2D6 were relatively weak (8.6% and 11.6% inhibition for CYP3A4 and CYP2D6, respectively). Our experimental data thus supports the results reported by Henderson *et al.*

The herbs Shokyou and Kankyou as they are known in Japanese, are derived from *Zingiber officinale* Roscoe, and both herbs are referred to as Ginger in English. In Kampo medicines, the herbs are clearly distinguishable by differences in efficacy based on the preparation of the extracts. *Zingiber officinale* dried directly is referred to as Shokyou, or as Kankyou if dried by steam heating. Shokyou and Kankyou differed remarkably with respect to their inhibitory effects on CYP3A4 and CYP2D6 (Kankyou > Shokyou). The chemical stability of their inhibitory component during the preparation process may be the main cause of the differences in inhibitory effects. The herbs known as Kanzo and Shakanzo in Japanese originate from *Glycyrrhiza uralensis* Fischer, *Glycyrrhiza glabra* Linne, and other herbs from the same genus, and are known as Licorice Root in English. In Kampo medicines, these herbs are also distinguishable by differences in efficacy based on the preparation of extract. Kanzo and Shakanzo were similar to each other in their inhibitory effects on CYP3A4 and CYP2D6.

The inhibitory effects of the methanol soluble and diethyl ether soluble fractions of several herbal extracts on CYP3A4 and CYP2D6 were compared. From the results, herbal extracts were classified into 2 groups: one group having the same inhibitory effect on CYP3A4 or CYP2D6 between the methanol soluble and diethyl ether soluble fractions, and the other showing a marked difference between the methanol and diethyl ether soluble fractions (the methanol soluble fraction > the diethyl ether soluble fraction). Angelica Dahurica Root, Incised Notopterygium Rhizome, Sappan Wood, and Scutellaria Root were classified in the former group, suggesting that inhibitory components against CYP3A4 and CYP2D6 are extractable with diethyl ether in neutral conditions, i.e., relatively non-polar components. Cassia Bark, Ephedra Herb, and Gambir Plant were classified into the latter group, suggesting that the components which inhibit CYP3A4 and CYP2D6 are relatively polar.

In conclusion, this *in vitro* study indicates that Sappan Wood, Rhubarb, Schisandra Fruit, Incised Notopterygium Rhizome, Angelica Dahurica Root, and other herbs inhibit human liver microsomal erythromycin *N*-demethylation, a marker of CYP3A4 activity. In addition, Phellodendron Bark, Coptis Rhizome, Sinomenium Stem, Sappan Wood, and Rhubarb, and other herbs inhibit human liver microsomal dextromethorphan *O*-demethylation, a marker of CYP2D6 activity. The prediction of herb-drug interactions in clinical situations should be possible through the identification of the components which are responsible for inhibition of liver microsomal CYP-mediated drug metabolism and by the clarification of the inhibitory mechanisms and components of each herb. Several herbal components responsible for inhibition of liver microsomal CYP-mediated drug metabolism are currently being identified and the results of these studies will be reported elsewhere.

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Japanese abstract

78種の生薬エキスについて，ヒト薬物代謝酵素シトクロム P450 3A4（CYP3A4）および P450 2D6（CYP2D6）に対する阻害作用を調べた。生薬エキス粉末からメタノール可溶性画分を調製し，添加量 1.65 mg/mL で反応を行った。CYP3A4 の指標として用いたヒト肝ミクロソーム中のエリスロマイシン *N*-脱メチル化活性に対して 50％以上の阻害を示した生薬は 31 種であり，その中の 8 種（白芷，桂皮，丁子，羌活，牡丹皮，大黄，蘇木，五味子）が阻害率 90％以上を示した。CYP3A4 活性に対する阻害の強さは，蘇木，大黄，五味子，羌活，白芷の順であり，IC_{50} 値は，それぞれ 43, 77, 127, 144 および 185 μg/mL であった。一方，CYP2D6 の指標として用いたヒト肝ミクロソーム中のデキストロメトルファン *O*-脱メチル化活性に対して 50％以上の阻害を示した生薬は 28 種であり，その中の 13 種（桂皮，丁子，黄連，麻黄，釣藤鉤，羌活，厚朴，牡丹皮，黄柏，大黄，蘇木，防已，山椒）が阻害率 90％以上を示した。CYP2D6 活性に対する阻害の強さは，黄柏，黄連，防已，蘇木，大黄の順であり，IC_{50} 値は各々 4, 14, 40, 52 および 64 μg/mL であった。これらの結果から，CYP3A4 あるいは CYP2D6 に対する阻害活性を示す生薬が複数存在することが明らかとなった。

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