Botanical-Drug Interactions: A Scientific Perspective

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- botanical products
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Abstract

There is a continued predisposition of concurrent use of drugs and botanical products. A general lack of knowledge of the interaction potential together with an under-reporting of botanical use poses a challenge for the health care providers and a safety concern for patients. Botanical-drug interactions increase the patient risk, especially with regard to drugs with a narrow therapeutic index (e.g., warfarin, cyclosporine, and digoxin). Examples of case reports and clinical studies evaluating botanical-drug interactions of commonly used botanicals in the US are presented. The potential pharmacokinetic and pharmacodynamic bases of such interactions are discussed, as well as the challenges associated with the interpretation of the available data and prediction of botanical-drug interactions. Recent FDA experiences with botanical products and interactions including labeling implications as a risk management strategy are highlighted.

Abbreviations

ADME:	absorption, distribution, metabolism, and excretion
AhR:	aryl hydrocarbon receptor
AUC:	area under the plasma concentration-time curve
BCRP:	breast-cancer resistant protein
DHB:	6′,7′-dihydroxybergamottin
CAR:	constitutive androstane receptor
C_{max}:	maximum plasma concentration
C_{min}:	trough plasma concentration
CL/F:	apparent oral clearance
CYP:	cytochrome P450
FDA:	Food and Drug Administration
GFJ:	grapefruit juice
K_i:	inhibition constant
MRP:	multidrug resistance associated protein
P-gp:	P-glycoprotein
PXR:	pregnane X receptor
PBPK:	physiologically-based pharmacokinetic
OATP:	organic anion-transporting polypeptide
SJW:	St. John's wort
SULT:	sulfotransferase
UGT:	UDP-glucuronosyl transferase

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Introduction

The use of botanicals as dietary supplements has increased significantly over the past decades. The botanical product sales in the US have increased steadily over the years, by 8% in 2007 over the preceding year, then increasing by 7% in 2008 and 14% in 2009 [1]. Recent surveys reveal that approximately 20% of Americans use botanicals and 20–30% indicated concurrent use of botanicals with conventional drugs [2,3]. In addition, there is an underreporting of such use to health care practitioners [4]. As more consumers use botanicals to promote health or to manage various common chronic diseases, for which they often take prescribed drugs concomitantly, the likelihood of potential pharmacokinetic and/or pharmacodynamic botanical-drug interactions increase.

Although the efficacy of some botanicals has been documented [5], there is a concern regarding the perceived safety of these products [6], particularly with respect to knowledge on botanical-drug interaction potential and its clinical signifi-

cance [7]. Indeed, clinically significant botanical-drug interactions have been reported. St. John's wort (*Hypericum perforatum* L.), a popular botanical used in the management of mild or moderate depression, has been shown to adversely alter the pharmacokinetics of several prescribed drugs (e.g., cyclosporine [8] and irinotecan [9]) resulting in therapeutic failure. A recent review of published clinical evidence identified 34 prescription drugs with a potential for interaction with botanical products. Most of the drugs are administered in long-term regimens and include antiretroviral agents, immunosuppressants, cardiovascular and oncology drugs with many of them having a narrow therapeutic index [10].

The purpose of this review is to present an overview of common mechanisms of botanical-drug interactions using specific literature examples. The evaluation of botanical-drug interactions using *in vitro* approaches and clinical trials, as well as the challenges associated with the interpretation of the results are reviewed and discussed. Moreover, this work highlights the regulatory perspectives on botanical products, including the labeling implications for potential interactions.

Mechanisms of Botanical-Drug Interactions

As for any drug-drug interactions, both pharmacokinetic (PK) and pharmacodynamic (PD) mechanisms may be implicated in botanical interactions with prescribed or over-the-counter drugs.

Altered pharmacokinetics

The most commonly documented botanical-drug interactions affect PK by altering drug absorption, distribution, metabolism, and excretion (ADME). Changes in drug absorption may be mediated through modulation of intestinal uptake and efflux transporters, while changes in metabolism/excretion occur through modulation of hepatic/renal uptake and efflux transporters, and/or through inhibition/induction of metabolizing enzymes. Examples of altered drug distribution as a result of protein binding displacement by botanicals have not been reported. Tissue uptake transporters also play a role in drug distribution, thus modulation of these transporters by botanical constituents may affect plasma and tissue exposure. The primary mechanism of reported botanical-drug PK interactions is modulation of metabolizing enzymes and/or transporters in the intestine and liver.

Modulation of metabolizing enzymes: The human cytochrome P450 (CYP) family of enzymes, including CYP1A1/2, CYP2A6, CYP2B6, CYP2C8/9/19, CYP2D6, CYP2E1, and CYP3A4/5 is involved in the oxidative metabolism (phase I) of the majority of drugs used in clinical practice. CYP3A4 is the most abundant CYP in the liver and intestine. While it catalyzes the metabolism of 50–60% of current marketed drugs [11], CYP2C19 and CYP2D6 are the major metabolizing enzymes for 15% and 20% of drugs, respectively [12]. UDP-glucuronosyl transferases (UGTs) include the UGT1 and UGT2 families of enzymes and are responsible for glucoronidation of 35% of drugs metabolized by phase II enzymes [13].

Inhibition of enzymes can be classified into reversible and irreversible inhibition. While the competitive mechanism of reversible inhibition results in an almost immediate response, mechanism-based inhibition is characterized by a time- and concentration-dependent blockage [14]. The irreversible inhibition can completely inactivate the drug's metabolism and can persist even after the withdrawal of the botanicals since the recovery of enzyme activity requires *de novo* enzyme synthesis. Irreversible inhibition of CYPs has been demonstrated *in vitro* by diallyl sulfone (in garlic), glabridin (in licorice root), methysticin (in Kava) [15, 16], and silybin (in milk thistle) [17].

The grapefruit juice (GFJ) furanocumarins, 6′,7′-dihydroxybergamottin (DHB), bergamottin, and paradisins, are capable of inhibiting CYP activity, both reversibly and irreversibly, with *in vitro* inhibitory constants in the nanomolar to micromolar range [18–20]. Mechanism-based inhibition of CYP3A by DHB and bergamottin may explain the clinically observed irreversible loss of intestinal CYP3A protein, without altered CYP3A mRNA levels, after ingestion of GFJ [20, 21]. Examples of the variable content of these enzyme modulators in different grapefruit juice products sold in the US is demonstrated in **Fig. 1 A**.

GFJ is a unique CYP3A inhibitor since usual dietary consumption of GFJ inhibits only enteric, but not hepatic CYP3A activity. Clinical evidence has shown that concomitant GFJ ingestion increases the systemic exposure of orally administered CYP3A substrates which have low oral bioavailability due to extensive presystemic extraction by enteric CYP3A [22]. Examples of grapefruit-drug interactions are illustrated in **Tables 1** and **2**, and their impact on drug labeling is listed in **Table 1S**.

Inhibition of CYP3A activity is proposed as the mechanism of interaction between cyclosporine and berberine, an isoquinoline alkaloid of goldenseal (*Hydrastis canadensis*), a popular botanical used as a topical antimicrobial and for digestive disorders. Cyclosporine (CYP3A4/P-gp substrate) blood concentration was increased in healthy volunteers [23] and in renal transplant recipients [24] after coadministration of 0.3 g (single dose) or 0.2 g (tid, 3 months), respectively, of a goldenseal product. Evidence of goldenseal's inhibition potential against CYP3A4, CYP2D6, and CYP2E1 has been demonstrated *in vitro* [25–27]. Inhibitory effect of berberine on CYP3A4 was observed at lower incubation concentrations (0.3 and 1 µM) while an inductive effect was shown at a higher concentration (10 µM) [27, 28]. Further support of CYP modulation by goldenseal was provided in three clinical studies where CYP2D6 and CYP3A4/5 activity was decreased, while CYP1A2 remained unaltered [29–31] (**Tables 1** and **2**).

Induction defines any mechanism that results in increased concentration of catalytically active protein involved in drug metabolism and/or transport. The most common mechanism of induction is ligand-dependent biding and activation of nuclear receptors that function as gene transcription factors, such as AhR (aromatic hydrocarbon receptor), CAR (constitutive androstane receptor), and PXR (pregnane X receptor) [32, 33]. Induction is a regulated process which requires time to reach a higher steady-state protein level. Also, the transcriptional regulation of enzymes is commonly cell-type and tissue- and species-selective [12, 32].

Preclinical and clinical data have provided evidence of St. John's wort (*Hypericum perforatum,* SJW) inductive effect on several CYP isoforms, including CYP3A4, CYP2C19, and CYP2E1. Chronic exposure of human hepatocytes to hyperforin, but not hypericin, increased mRNA and protein expression, and CYP3A4 activity [34]. The increased CYP3A4 expression by SJW is mediated via PXR activation [35–37] and hyperforin, one of the main active constituents of SJW, is the most potent agonist for PXR. In one study, its binding affinity K_i was detected to be 27 nM [35].

Induction of intestinal and hepatic CYP3A4 by SJW did alter the bioavailability and clearance of concurrent drugs that are mainly, or partly, metabolized by CYP3A4. For example, long-term SJW treatment reduced the area under the concentration-time curve

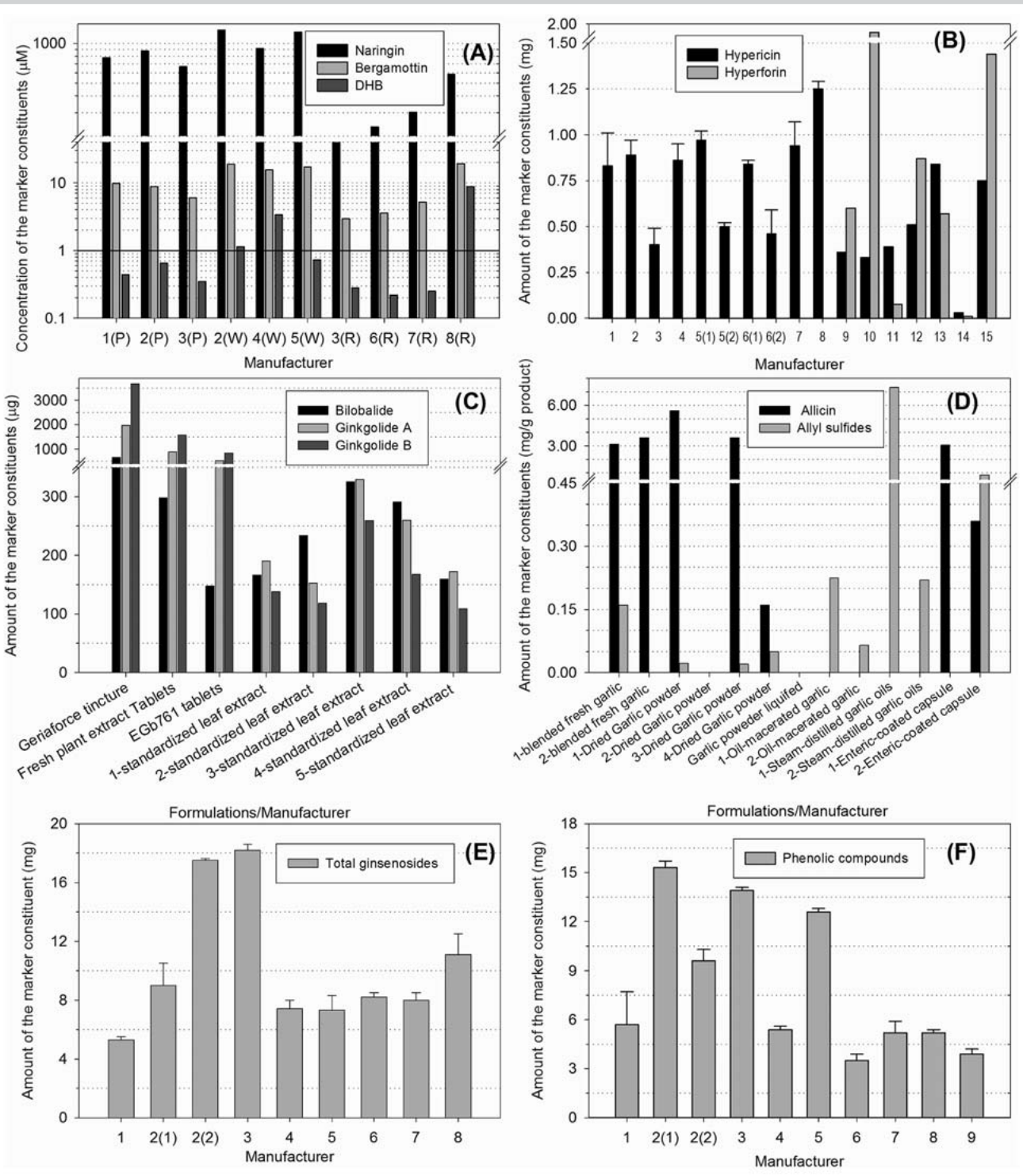

Fig. 1 Comparison of the variability of the marker constituents among different manufacturers (and different lots of the same manufacturer) or formulations of commonly used commercial botanical products in the US – grapefruit juice, St. John's wort, *Ginkgo biloba*, garlic, ginseng, and *Echinacea*. Bars represent the mean measured amount of the marker compound(s) listed in legends. Error bars (when available) depict the standard deviation of the average of two lots. Numbers in parentheses represent different formulations/preparations from the same manufacturer. **A** Grapefruit juice. Eight different brands of GFJ using three different varieties pink (P), white (W), and red (R) are demonstrated; from reference [194]. **B** St. John's wort (*Hypericum perforatum*); from references [54, 55]. **C** *Ginkgo biloba*. Four different formulations and five commercially available products from different manufacturers were evaluated; from references [195, 196]. **D** Garlic (*Allium sativa*). Six different formulations were evaluated from at least two different manufactures. Bars represent the range of the constituents measured. When a bar is not available, the constituents were not detected (below the limit of detection) in the product; from reference [106]. **E** Ginseng (*Panax ginseng*). Marker is reported as total ginsenosides; from reference [55]. **F** *Echinacea purpurea*. Marker is reported as total phenolic compounds, including echinacoside, cattaric acid, chologenic acid, and cichoric acid; from reference [55].

Table 1 Effect of botanical product extracts and their active constituents on metabolic enzymes and transporters.

Botanical (known enzyme/ transporter modulator)	Enzyme and transporter affected[a]	Possible affected drug class (drug example)[b]
Garlic extract (allicin and alliin)	CYP2D6 (↔) CYP3A4 (↔) CYP1A2 (↔) CYP2C9 (↔) P-gp (↑)	Antiretroviral (saquinavir) [97, 98], CYP2E1 probe chlorzoxazone [45, 46]
Garlic oil (diallyl sulfide)	CYP2E1 (↓)	
Ginkgo biloba [Flavonoids (e.g., quercetin, kaempferol) and terpenoids (ginkgolides A and B, and bilobalide)]	CYP2D6 (↔) CYP3A4 (↔) CYP2C19 (↑) CYP2C9 (possible ↓) P-gp (↓) *OATP2B1 (↓)*	Antihistamine (fexofenadine) [79, 197], beta-blocker (talinolol) [76, 77], proton pump inhibitor (omeprazole) [188]
Ginseng (Ginsenoside Rb1, Rb2, Rc, Rd, Re, and Rf)	CYP3A4 (↔) CYP2D6 (↔) CYP1A2 (↔) CYP2E1 (↔) *CYP1A1 (↓) CYP1B1 (↓) CYP2C19 (↓) CYP2C9 (↓)*	
Goldenseal (Alkaloids berberine and hydrastine)	CYP3A4 (↓) CYP2D6 (↓) CYP1A2 (↔) CYP2E1 (↔)	Central nervous system agent (midazolam) [31], CYP2D6 probe debrisoquine [29], immunosuppressant (cyclosporine) [23, 24]
Grapefruit juice (Flavonoids naringin/naringenin and quercetin, and furanocoumarins bergamottin, 6′7′-dihydroxybergamottin)	Enteric CYP3A4 (↓) P-gp (↓) OATP1A2 (↓) OATP2B1(↓)	Antihistamines (fexofenadine, terfenadine), anti-infectives (e.g., erythromycin, halofantrine, praziquantel), antiretrovirals (saquinavir), cardiovascular drugs (e.g., aliskiren, azelnidipine, celiprolol, felodipine, manidipine, nicardipine, nifedipine, nimodipine, nisoldipine, talinolol), central nervous system agents (e.g., alfentanil, buspirone, carbamazepine, diazepam, fluvoxamine, methadone, midazolam, phenytoin, sertraline, triazolam), immunosuppressants (e.g., cyclosporine, tacrolimus), statins (e.g., atorvastatin, lovastatin, simvastatin), oncology agents (etoposide) [22]‡
Milk thistle (Flavonolignans silybin)	CYP2C9 (↓) CYP3A4 (↔) CYP1A2 (↔) CYP2D6 (↔) CYP2E1 (↔) *CYP2C8 (↓) UGT1A6/9 (↓) UGT1A1 (↓) UGT2B7/15 (↓)* (OATP1B1↔) P-gp (↓) *MRP1 (↓)*	Cardiovascular drugs (e.g., losartan [169] and talinolol [198])
St. John's wort [Phloroglucinol hyperforin and flavonoids (e.g., quercetin)]	CYP1A2 (↑) CYP2E1(↑) CYP3A4 (↑) CYP2C9 (↑) CYP2C19 (↑) P-gp (↑) *UGT1A6 (↓)*	Antiretrovirals (e.g., indinavir, lamivudine, nevirapine), cardiovascular drugs (e.g., digoxin, ivabradine, nifedipine, talinolol, verapamil, warfarin), central nervous system agents (e.g., amitriptyline, alprazolam, buspirone, methadone, midazolam, phenytoin, sertraline), hypoglycaemic agents (gliclazide), immunosuppressants (e.g., cyclosporine, tacrolimus), statins (e.g., atorvastatin, simvastatin), oncology agents (imatinib, irinotecan), proton pump inhibitors (e.g., cimetidine, omeprazole,), respiratory system agents (e.g., fexofenadine, theophylline) [92, 93] ‡

[a] The enzymes and/or transporters modulating effect [(↑) increase, (↓) decrease, (↔) no effect] are based on human trials except those in *italic*, which are based on *in vitro* data only. [b] Listed drugs with published clinical drug-botanical interaction based on pharmacokinetic mechanism. ‡ References in brackets refer to comprehensive reviews of botanical-drug interactions with details of the respective clinical trials

(AUC) and/or maximum plasma concentration (C_{max}) of nevirapine [38], ivabradine [39], quazepam [40], and verapamil [41]. SJW (standardized extract, 0.825 mg hypericin, and 12.5 mg hyperforin, 3 tablets/day, 14 days) increased the oral clearance of both enantiomers of warfarin, 29% for S-warfarin (CYP2C9 substrate) and 23% for R-warfarin (CYP3A4/CYP1A2 substrate), resulting in a significant reduction of the anticoagulant effect of rac-warfarin [42].

A dose-dependent effect of SJW against CYP2C19 and CYP2E1 was observed in human hepatocytes; inhibition at a low incubation concentration (8 µg/mL) and induction at a higher concentration (800 µg/mL) [43]. Also, CYP1A2 protein expression raised 2%, 30%, and 90% by increasing concentrations of SJW extracts (100-, 10-, and 1- fold dilution of 9.4 mM of hypericin and 10 mM hyperforin) in human intestinal cells (LS180) [44]. In humans, the inductive effect of SJW (900 mg/day, 14 days) on CYP2E1 and CYP2C19 was demonstrated by the increase in the serum metabolic ratio of clorzoxazone [45, 46] and in the urinary excretion of mephenytoin metabolite [47], respectively. Additionally, SJW induced both CYP3A4-catalyzed sulfoxidation and CYP2C19-dependent hydroxylation of omeprazole resulting in a CYP2C19 genotype-dependent decrease in omeprazole AUC and C_{max} [48] (**Table 2**). SJW constituents also likely mediated the induction of CYP2C9 metabolism (and/or CYP2C19) of gliclazide (47% increase in the oral clearance) leading to a reduction in the drug's AUC and half-life [49]. On the other hand, short-term and long-term SJW coadministration failed to alter the PK of tolbutamide, a CYP2C9 substrate, in two PK studies [50, 51]. Induction of CYP1A2 (20% increase in the metabolic ratio of caffeine) was observed only in the female population using SJW (300 mg tid, 14 days) [52] in one study whereas no effect was observed in three other trials [45, 46, 50]. Interestingly, theophylline plasma concentration was decreased in a female patient after SJW administration [53]. The inconsistency in the clinical observations may be explained by the variability on the content of SJW components found among products [54, 55] including the constituent of the botanical that is responsible for enzyme induction, hyperforin (**Fig. 1 B**).

Additionally, botanicals such as SJW may exhibit a biphasic effect on CYP enzymes: an initial inhibitory effect when given 24 hr pri-

Table 2 Selected examples of clinical botanical-drug interactions involving drugs that are CYP, P-gp, and OATP substrates.

Botanical product (study design)	Prescribed/probe drug (dosage)	Clinical interaction outcome (possible mechanism)	Reference
Echinacea purpurea (500 mg extract, tid, 28 days, n = 8 male and 5 female healthy volunteers)	Midazolam (8 mg SD)	27% ↓ in AUC and 37% ↑ in CL/F (induction of CYP3A)	[199]
Ginkgo biloba (standardized extract with 23% flavonol glycosides and 7% terpene lactones, 140 mg bid, 12 days, n = 6 CYP2C19 homozygous EM, n = 5 heterozygous EM, n = 7 PM healthy Chinese males)	Omeprazole (40 mg SD)	In PM, 25% ↓ in AUC metabolic ratio. In homozygous and heterozygous EM, 58% ↓ and 47% ↓ in AUC metabolic ratio, respectively (genotype-dependent induction of CYP2C19)	[188]
Ginkgo biloba (standardized extract, 120 mg tid, 14 days, n = 12 male healthy volunteers)	Talinolol (100 mg SD)	21% ↑ in AUC and 33% ↑ in C_{max} (inhibition of Pg–p)	[76]
Ginkgo biloba (standardized extract, 120 mg tid, 24 days, n = 10 male Chinese healthy volunteers)	Talinolol (100 mg, days 9–23)	26% ↑ in AUC and 36% ↑ in C_{max} (inhibition of Pg–p)	[77]
Goldenseal (as berberine 0.2 g tid, 3 months, n = 52 renal-transplant recipients)	Cyclosporine (3 mg/kg bid, 3 months)	29% ↑ in C_{min} (inhibition of CYP3A)	[24]
Goldenseal (900 mg root extract, tid, 28 days, n = 6 male and n = 6 female healthy volunteers)	Midazolam (8 mg SD)	40% ↓ in serum metabolic ratio (inhibition of CYP3A)	[29]
Goldenseal (1227 mg root extract with 77 mg of berberine, and 132 mg of hydrastine, tid, 14 days, n = 8 male and n = 8 female healthy volunteers)	Midazolam (8 mg SD)	62% ↑ in AUC, 41% ↑ in C_{max}, and 36% ↓ in CL/F (inhibition of CYP3A)	[31]
Goldenseal (900 mg root extract, tid, 28 days, n = 6 male and n = 6 female healthy volunteers)	Debrisoquine (5 mg SD)	40% ↓ in urinary recovery ratio (inhibition of CYP2D6)	[29]
Grapefruit juice #(200 ml double-strength*#, tid for 2 days, day 3: 200 ml with simvastatin single dose and at 30 and 90 min postdose, n = 10 healthy volunteers) **or** §(8 oz. (237 mL) single-strength*§ morning for 3 days, simvastatin dosed in the evening day 3, n = 16 healthy volunteers)	Simvastatin (#60 or §20 mg SD)	#1500% ↑ or §90% ↑ in AUC of simvastatin, and #580% ↑ or §30% ↑ in AUC of simvastatin acid; #840% ↑ or §80% ↑ in C_{max} of simvastatin, and #550% ↑ or §30% ↑ in C_{max} of simvastatin acid (inhibition of enteric CYP3A4)	[200]# [201]#§
Grapefruit juice (200 ml normal strength, tid for 5 days, day 3: 200 ml with aliskiren dose in the morning and at 4 and 12 hours postdose, n = 5 female and n = 6 male healthy volunteers)	Aliskiren (150 mg SD)	81% ↓ in C_{max}, 61% ↓ in AUC (inhibition of OATP2B1)	[202]
Kava (1000 mg root extract, bid, 28 days, n = 6 male and n = 6 female healthy volunteers)	Chorzoxazone (250 mg SD)	40% ↓ in serum metabolic ratio (inhibition of CYP2E1)	[29]
St. John's wort (600 mg extract, qd, 14 days, n = 11 renal transplant patients)	Cyclosporine (median 2.8 mg/kg/day)	46% ↓ in AUC, 42 ↓ in Cmax 41% ↓ in C_{min}; Cyclosporine dose adjustment was required to ensure concentrations are within the therapeutic range (induction of CYP3A and P-gp)	[87]
St. John's wort (300 mg extract, tid, 14 days, n = 6 CYP2C19*1/*1, n = 4 CYP2C19*2/*2 and n = 2 CYP*2/*3 healthy males)	Omeprazole (20 mg qd, 14 days)	44% ↓ and 38% ↓ in AUC of omeprazole, 37% ↑ and 0% in AUC of 5-OH-omeprazole, 136% ↑ and 159% ↑ in AUC of omeprazole sulfone in subjects with CYP2C19 wild-type and variant, respectively. (induction of CYP3A and CYP2C19)	[48]

Abbreviations: C_{max}, maximum plasma concentration; C_{min}, trough plasma concentration; AUC, area under the plasma concentration-time curve; SD, single dose administration; EM, extensive metabolizers; PM, poor metabolizers; CL/F, apparent oral clearance; ↑ increase ↓ decrease. *# Double-strength: one can of GFJ frozen concentrate diluted with one can of water. *§ Single-strength: one can of GFJ frozen concentrate diluted with 3 cans of water

or to testing with induction after chronic exposure [34]. Using CYP recombinant systems, hyperforin behaved as a competitive inhibitor of CYP3A4 (K_i = 0.48 µM) and CYP2C9 (K_i = 1.8 µM) and a noncompetitive inhibitor of CYP2D6 (K_i = 1.5 µM). I3,II8-biapigenin, a flavonoid component of SJW, was also shown to be a competitive inhibitor of CYP3A4 (K_i = 0.038 µM), CYP2C9 (K_i = 0.32 µM), and CYP1A2 (K_i = 0.95 µM) [56]. The acute inhibitory potential of SJW constituents was confirmed in another *in vitro* study [34, 57]. In humans, single-dose exposure of SJW caused inhibition of voriconazole (CYP2C19/CYP2C9/CYP3A4 substrate) metabolism, while long exposure led to induction [58]. Details of this and other examples of SJW-mediated interactions and drugs that are likely to interact with SJW are discussed throughout this work and listed in **Table 1** and **Table 1S**.

Interactions resulting from *Ginkgo biloba* modulation of CYP2C9 function remain controversial. Ginkgo extract competitively inhibited CYP2C9 (K_i = 14.8 µg/mL) in human liver microsomes [59]; but in clinical trials, coadministration of the standardized ginkgo leaf extract (EGb-761), given either as 80 mg/day or 240 mg/day for 3 or 7 days, had no effect on the PK of the CYP2C9 substrates tolbutamine, diclofenac [59], warfarin [60], and ticlopidine [61] in healthy subjects. However, using a higher dose (360 mg/day) for a longer period (28 days) than those used in the previous studies, the AUC of tolbutamide was decreased by 16% in healthy volunteers, possibly indicating CYP2C9 induction [62]. Again, the disagreement among studies may also be due to the variable content of the active constituents that may be found in different ginkgo formulations as exemplified in **Fig. 1C**.

Compared to CYP enzymes, evidence of the modulation of phase II enzymes by botanicals is limited. Ginkgo extract increased glutathione S-transferase (GST) expression and activity levels in HepG2 and Hep1c1c7 cells lines [63] and in rodents after 1 week of exposure [64]. Ginkgo extract and its main flavonoids, quercetin and kaempferol, may also modulate UGT enzymes as demonstrated by the inhibition of the glucuronidation of mycophenolic acid in human microsomes; while ginkgo terpenoids, ginkgolides A and B, and bilobalide, showed no effect [65].

Silybin, an active flavonolignan of milk thistle (*Silybum marianum*), was shown to be a potent inhibitor of UGT1A1 (IC_{50} of 1.4 µM) and other UGTs with less potency (IC_{50} range = 28–75 µM) [17]. Silybin also inhibited CYP3A4 activity *in vitro* (K_i range = 5–160 µM) [17]. However, the modulation of these enzymes has not been observed *in vivo*. Administration of milk thistle extract (standardized 80% of silymarin, 200 mg tid, for 4 or 12 days) did not affect the PK of irinotecan (CYP3A4/UGT1A1 substrate) in six cancer patients. This appears to be consistent with the low levels of the components with enzyme modulation activity achieved *in vivo*. The range of the maximum plasma concentration of silybin was 0.0249 to 0.257 µM [66], which is below the *in vitro* inhibition constants of UGT1A1 and CYP3A4.

Modulation of transporters: Recently, many human drug transporters have been identified (**Fig. 1S**) and P-glycoprotein (P-gp), expressed by the MDR1 gene, is the best characterized one. P-gp functions as an efflux transporter in several tissues including the gastrointestinal tract, liver, kidney, and blood-brain barrier. Inhibition or induction of P-gp (regulated by PXR or CAR [67,68]) by interacting drugs or botanical constituents may alter drug ADME resulting in significant pharmacokinetic consequences.

The inductive effect of SJW on P-gp appears to be the mechanism of interaction with known P-gp substrates. The C_{max} and/or AUC values of digoxin [69,70], talinolol [71], and fexofenadine [72] were decreased after long-term administration of SJW. Supportive evidence of P-gp induction by longer SJW exposure is based on studies conducted *in vitro* [73,74] and in humans [75]. A 1.4-fold increase in intestinal P-gp expression was observed after a 14-day administration of SJW in healthy volunteers [75]. The induction of P-gp by SJW appears to be mainly attributed to hyperforin [74].

Ginkgo biloba extract (120 mg tid for 14 or 28 days) increased the C_{max} and AUC of talinolol in healthy volunteers, probably by inhibition of P-gp-mediated efflux (**Tables 1** and **2**) [76,77]. On the other hand, ginkgo extract (120 mg bid, 28 days or 80 mg tid, 7 days) failed to change the PK of digoxin [78] and fexofenadine [79]. In Caco-2 cells, ginkgo extract inhibited digoxin efflux with an IC_{50} of 24 µg/mL [80], and its flavonoids quercetin, kaempferol, and isohamnetin were shown to be P-gp substrates with inhibitory and inductive effect on P-gp [81].

Organic anion-transporting polypeptides (OATPs) are a family of transporters distributed throughout the body, including sites relevant to drug ADME (**Fig. 1S**). OATPs have been increasingly recognized by their contribution to drugs pharmacokinetic behavior. In the small intestine, OATP1A2 and OATP2B1 are the main OATP transporters.

Suggestion of OATPs inhibition by botanicals is demonstrated by the 85%, 56%, and 82% decrease of OATP2B1-mediated uptake of estrone-3-sulfate by ginkgo extract [82], *Echinacea purpurea* extract [82], and 5% GFJ [83], respectively, in transfected HEK293 cells. Additional data indicate that flavonoids in GFJ may be responsible for the OATP inhibition. Naringin (50 µM) inhibited OATP1B1- and OATP1A2-mediated uptake of dehydroepiandrosterone sulfate [84] and fexofenadine [85], respectively; while its aglycone naringenin (10–50 µM) inhibited OATP1B1- and OATP2B1-mediated influx of dehydroepiandrosterone sulfate [84] and glibenclamide [83]. Clinically, the ingestion of naringin (1210 µM aqueous solution or 1234 µM in GFJ) or GFJ resulted in 22 to 42% reduction in fexofenadine AUC; while a GFJ product, reported to be rich in furanocumarins, did not alter fexofenadine exposure [85]. Other OATP substrates such as etoposide, celiprolol, and aliskiren (**Table 1**) had their AUC reduced (between 85–26%) by the co-intake of GFJ [22].

Metabolism-transport interplay: In a 2007 review, 29% (10 out of 34) of commonly prescribed drugs with clinical evidence of botanical interaction were identified as substrates of P-gp. These include cyclosporine, digoxin, fexofenadine, imatinib, indinavir, irinotecan, nevirapine, simvastatin, saquinavir, and tacrolimus [10]. Interestingly, except digoxin and fexofenadine, these drugs are also substrates for CYP3A4. Thus, it appears that a dual substrate for CYP3A4 and P-gp has a much higher potential for interaction with botanicals. As CYP3A4 and P-gp play a role in limiting drug bioavailability after oral administration, the interdependence of metabolism and transport processes may represent a potentially important mechanism of interaction. The observed decrease of trough blood concentrations of cyclosporine, in renal or heart transplant recipients with long-term coadministration of SJW, appears to be due to an increase in both CYP3A4 activity and P-gp intestinal efflux. This botanical-mediated PK interaction was associated with the transplant graft rejection observed in all reported cases [8,86,87]. Similar mechanism of SJW interaction was postulated for imatinib [88,89], irinotecan [9], indinavir [90], and simvastatin [91]. An update of SJW-mediated clinical drug interactions can be found in comprehensive reviews [92,93].

The "metabolism-transport interplay" may pose a challenge in the prediction and assessment of the specific role played by each of these mechanisms in the altered exposure of coadministered drugs [94]. For instance, assessment of preclinical and clinical data of garlic (*Allium sativum*) CYP3A4 and P-gp modulation raised the hypotheses of garlic affecting hepatic and intestinal enzyme-transporter interplay leading to pharmacokinetic interactions. *In vitro*, acute exposure of the mixture of constituents of different garlic formulations (fresh, dried garlic powder, oil, and aged-garlic extract) inhibited CYP3A4 and moderately inhibited P-gp activity [25]. Allicin (diallyl thiosulfinate), found in crushed fresh garlic, inhibited CYP3A4 activity and ritonavir P-gp-mediated efflux in Caco-2 cells [95]; while garlic diallyl sulfide showed no inhibitory effect on P-gp [96]. In healthy volunteers, the C_{max}, C_{min}, and AUC of saquinavir (CYP3A4/P-gp substrate) were reduced 51%, 54%, and 49%, respectively, after garlic use (dried garlic powder GarliPure®, 4.64 mg allicin and 11.2 mg alliin, bid, 21 days). After a 10-day washout, the C_{max}, C_{min}, and AUC values were still at 60–70% of the baseline values [97]. It has been suggested that induction of CYP3A4 and/or P-gp by garlic components may represent the underlying mechanism of the altered saquinavir PK. In another interaction study in healthy volunteers, garlic supplements (using a product from the same manufacturer and dosing regimen as described above) led to a 15% decrease of saquinavir AUC [98]. To explore the causal mechanisms, the investigator further determined the expression of P-gp and CYP3A4 proteins in the duodenum and the hepatic CYP3A4 function (by erythromycin breath test). Garlic administration increased the intestinal P-gp protein levels by 31%, yet no differences in the levels of CYP3A4 in the intestine and the CYP3A4 function in the liver were found. A negative correlation between changes in duodenal P-gp expression and the bioavailability of saquinavir was observed. Moreover, garlic did not alter the clearance of the CYP3A4 substrate simvastatin [98]. No effect on CYP3A4 function, evaluated by changes in the metabolic ratios of alprazolam [83] and midazolam [45,46], was also observed with the use of Kwai garlic supplements (standardized on allicin potential for 14 days) or garlic oil (5 days), respectively. In contrast, no change of ritona-

vir exposure was observed after short-term (4 days) consumption of garlic extract (Natural Source Odourless Garlic Life®, < 50 µg/g of allicin, 10 mg bid) by healthy volunteers [99].

These results highlight several confounding factors in the interpretation of botanical-drug interaction. First, preclinical models may not predict clinical outcomes. *In vitro,* aged-garlic extracts, low in alliin but rich in flavonoids, reduced saquinavir efflux by P-gp and/or MRP2 in liver models (HepG2 cells and rat liver slices) resulting in increased intracellular concentration with opposite effect (increased efflux) for darunavir [100]; whereas aged-garlic extract increased the efflux of saquinavir and darunavir in Caco-2 cells but decreased CYP3A metabolism in the rat jejune [101]. In rats, the administration of garlic oil for 5 days increased mRNA and protein levels of CYP3A1, CYP1A1, and CYP2B1 [102, 103]. The mechanism may be through activation of rat CAR [104], which regulates the expression of enzymes and drug transporters (P-gp) in the liver and intestine [33], by diallyl sulfide in garlic oil [104]. Conversely, exposure of human hepatocytes to garlic extracts (0–200 µg/mL) had no effect on CYP3A4 activity [105]. In combination, these results may suggest that there are interspecies differences in the regulation of CYP3A/P-gp orthologues between rodents and humans [98]. Second, a negative interaction outcome from short-term administration in humans may not represent what may be observed with prolonged use in a clinical setting. For example, the duration of garlic therapy in the ritonavir study (4 days) may have been too short to observe a significant P-gp inductive effect. Third, the composition of garlic active constituents is highly variable among different formulations (**Fig. 1 D**) [106, 107]. In addition, extrapolation of the content of dried garlic powder to fresh garlic is inappropriate as the biotransformation of allicin from dried garlic supplements is extremely erratic, with the oral bioavailability of allicin varying between 5 to 95% among formulations [107]. The formulations used in the assessment of potential interactions associated with their components specific activity towards enzymes and transporters may lead to different modulating effects and ultimately different *in vivo* outcomes. The identification and standardization of the active constituents of botanical products are therefore essential to the evaluation of botanical-drug interactions.

Additional examples of pharmacokinetic-based interactions as a result of inhibition or induction of CYP enzymes and/or transporters are listed in **Table 1** (summary of selected clinical studies can be found in **Table 2**). Overall, the presented examples have demonstrated that botanicals may act as modulators of drug-metabolizing enzymes and/or transporters and may have an impact on the PK of coadministered drugs which are mainly eliminated by the affected enzyme or transporter.

Altered pharmacodynamics

Pharmacodynamic interactions, although less common, can also occur, resulting in either an augmented or attenuated response. If the effect of the botanical on the coadministered drug is enhanced (e.g., by synergistic or additive effect of the drug and botanical on the same drug targets), adverse events/toxicity may occur. By contrast, some botanicals may contain compounds with antagonistic properties, which are likely to reduce drug efficacy and cause therapeutic failure.

The most commonly reported pharmacodynamic botanical-drug interactions involve antithrombotic drugs since many commonly used botanicals possess anticoagulant, antiplatelet, and/or fibrinolytic properties. In a survey of 250 patients using antithrombotic therapy, 76 were taking botanical supplements for the last 12 months. Twenty-three were taking one or more of the botanical products – Asian ginseng (*Panax ginseng*), garlic, and ginkgo – with eight of those patients being at risk of potential botanical-drug interactions. Interestingly, 90% (n = 225) of the patients did not disclose the use of botanical supplements to their health care practitioners [108]. This example highlights the need for better education and actions to encourage communication among clinicians and patients about botanical use.

Botanicals such as *Ginkgo biloba* have demonstrated the potential for clinical antiplatelet activity due to the inhibition of platelet activating factor by ginkgolides [109]. Several patient cases of spontaneous bleeding (usually intracranial, intra-ocular, or post-operative) as a result of gingko use alone [110] or potential ginkgo interactions with warfarin and aspirin have been reported [111]. However, in two clinical trials, ginkgo standardized extract (EGb 761, 240 mg/day for 7 days or 100 mg/day for 4 weeks) failed to alter the PK and PD of warfarin in healthy subjects [60], and in patients stabilized on long-term warfarin [112]. Likewise, the concomitant use of EGb 761 (300 mg/day, 4 weeks) and aspirin did not result in enhanced inhibition of platelet function and coagulation in elderly patients at risk of cardiovascular disease [113]. Pharmacodynamic interaction studies of EGb761 with either clopidogrel or cilostazol in healthy subjects reported a non-significant additive effect on antiplatelet activity; although EGb761 did enhance the bleeding time prolongation effect of cilostazol [114]. It is noteworthy that the systemic levels of ginkgo active constituents were not provided in the above studies. In a PK study, the C_{max} values of ginkgolide A were 9.4 and 42.9 ng/mL after oral intake of EGb 761 by elderly (120 mg) and healthy volunteers (240 mg), respectively; whereas the C_{max} values of ginkgolide B were 6.2 and 18.2 ng/mL in elderly and healthy volunteers, respectively [115]. These systemic levels were lower than the platelet activating factor-IC_{50} values of 15.6 and 3.5 µg/mL of gingkolides A and B, respectively [116]. However, the marked difference of ginkgolides content among different ginkgo formulations and commercial products (**Fig. 1 C**) makes future extrapolation of clinical outcomes difficult.

The effect of warfarin was decreased by the coadministration of Asian ginseng *(Panax ginseng*) [117]. Ginseng has the potential to interfere with the coagulation cascade and therefore interact with warfarin. However, no pharmacodynamic interactions with warfarin and *P. ginseng* were observed in healthy subjects [42], patients with cardiac valve replacement [118], or with ischemic stroke [119]. The use of a different species of *Panax, P. quinquefolius* (American ginseng), in contrast, reduced the anticoagulant effect of warfarin in healthy adults [120]. Chemically several differences have been demonstrated between *P. ginseng* and *P. quinquefolius,* including the presence of the active constituents ginsenoside Rf in *P. ginseng* versus pseudoginsenoside F11 in *P. quinquefolius* [121]. Variation on total ginsenoside content among different commercial products of *P. ginseng* was also observed (**Fig. 1 E**).

Garlic could also potentially increase the effect of anticoagulant drugs as allicin has demonstrated antiplatelet activity [122]. To date, two cases have been reported in patients stabilized on warfarin who experienced doubling of international normalization ratio (INR) and had an increase in clotting time after garlic consumption (no details on dosing regimen) [123]. Similarly, there have been several reports of increased INR in patients taking warfarin and dong quai (*Angelica sinensis*), which also inhibits platelet aggregation; though no bleeding episodes were observed [124].

Potential pharmacodynamic interactions with antithrombotic drugs may occur as result of PK modulation. For example, SJW (300 mg tid, 14 days) increased the platelet inhibition effect of clopidogrel in hyporesponsive volunteers and patients via induction of CYP3A4 metabolic activity as measured by the erythromycin breath test [125]. Whether the activity of CYP2C19 was also modulated by SJW, and the contribution of CYP2C19*2 polymorphism on the response of the studied population were not evaluated.

While some botanicals have an additive effect on the pharmacological action of anticoagulants, coenzyme Q10 has demonstrated an antagonistic interaction with warfarin. A decrease in INR values below 2 was observed in three elderly patients who were stabilized on warfarin and concomitantly using 30 mg/day of coenzyme Q10 (ubidecarenone). The structural similarity of coenzyme Q10 to vitamin K_2 may suggest the enhanced coagulation effect of the coenzyme Q10 [126].

Overall, the impact of botanical supplements on normal hemostasis and antithrombotic therapy should be given careful consideration. Gingko and garlic are examples of botanicals in which precautionary use is recommended in anticoagulants/antiplatelet drug labels as it might pose an additive risk of bleeding, whereas ginseng and coenzyme Q10 may have an antagonist effect [127].

Cases of pharmacodynamic interactions with botanicals and drugs acting on the central nervous system (CNS) have been widely reported. For example, concurrent use of SJW, the most commonly used botanical antidepressant [128], with serotonergic drugs, such as selective serotonin-reuptake inhibitors (e.g., sertraline [129], fluoxetine [130], paroxetine [131], and nefazodone [132]) and serotonin agonists (e.g., buspirone [133]), caused manic episode or serotonin syndrome in some patients. The probable mechanism of interaction was an additive serotonergic effect due to the inhibitory activity of SJW on serotonin re-uptake transporters in the CNS [134].

Kava (*Piper methysticum*), a popular botanical with anxiolytic and sedative properties, has demonstrated additive effect with the benzodiazepine levodopa [135]. The observed increase in the duration and number of "off" periods in Parkinson's patients may be attributed to the dopamine antagonistic effect of kava extract and/or kavalactones [136]. Besides the synergistic effect at gamma-aminobutyric acid (GABA) receptors, inhibition of drug-metabolizing enzymes, such as CYP3A4 [137,138] may explain the enhanced CNS-depressant effect of alprazolam resulting in a semicomatose state when kava was taken concomitantly [139]. The FDA has issued a consumer advisory about kava use [140] in the face of safety concerns that have been raised about kava hepatotoxicity [141].

Other examples of adverse events as a result of botanicals-CNS drugs pharmacodynamic interactions include reports of a patient manic episode after concomitant use of phenelzine and ginseng [142] and a possible PK/PD interaction of ginkgo with trazodone which led to an elderly patient's comatose state [143].

Although most pharmacodynamic interactions reported in the literature and reviewed in this work focused on adverse events, not all interactions will result in an undesirable effect. For example, investigators of a clinical trial claimed beneficial effects of milk thistle extract (140 mg tid, 3 months), a strong antioxidant with iron chelating activity, in combination with desferrioxamine. The combined botanical-drug therapy was more effective than desferrioxamine alone in reducing serum ferritin levels in patients with beta-thalassemia major. It was suggested that silymarin and desferrioxamine could be used safely and effectively in the treatment of iron-loaded patients [144].

There was also a report of an interaction between chlorpropamide and karela (*Momordica charantia*), a fruit widely consumed in Asia, Africa, and the Caribbean and used for management of type 2 diabetes mellitus as an Ayurvedic medicine. A patient whose blood glucose levels was poorly controlled when taking chlorpropamide alone, experienced a better outcome with coadministered karela [145]. Many botanicals such as *Salacia oblonga* [146] and *Cajanus cajan* [147] have shown hypoglycemic effects in animal models and in a limited number of studies in healthy adults. Since hypoglycemic agents tend to have additive effects when taken concomitantly, the potential for interaction affecting diabetic control and resulting in serious adverse events is high.

The clinical implications of any drug interactions depend on a variety of patient-dependent factors such as age, sex, nutrition habits, health status, genetic factors and metabolizing capacity, coadministered drugs and applied dosage regimens. Botanical- dependent factors include the species, dosing regimen, and composition of active constituents [148]. Nonetheless, altered pharmacokinetics almost inevitably leads to a significant change in response of a narrow therapeutic index-drug possibly resulting in serious or life-threatening adverse effects.

Evaluating Botanical-Drug Interaction

Considerable effort has been focused on understanding and predicting the modulation of enzymes and transporters *in vivo*. A collection of gene- and protein-based assays has been used to identify the specific phase I/II enzymes and transporters involved in drug ADME, and ultimately, predict potentially significant drug interactions. Identification of the extent of metabolism by a specific enzyme and its affinity by a specific transporter is required for all new drugs by the FDA. In addition, the potency of enzyme inhibition or induction and transporter inhibition is required to be quantified [149]. These *in vitro* techniques may also be useful to provide mechanistic information and better characterize the active components of botanicals that have potential for drug interaction.

In silico mechanistic models, such as physiologically-based pharmacokinetic (PBPK), may be a useful tool for the prediction of potential botanical-drug interactions and evaluation of various clinical situations, including the existence of multiple patient factors (complex scenarios) such as genetic polymorphisms of certain metabolizing enzymes and disease states. PBPK simulations may also offer valuable insight into optimizing the design of interaction trials. Besides *in vitro* drug interaction data, sufficient information of clinical pharmacology (e.g., ADME) of both the victim drug and the enzyme/transporter modulators of botanicals need to be collected to develop PBPK models [150]. One example of PBPK application in this area is the investigation of GJF-mediated interactions with CYP3A substrates. The impact of intestinal CYP3A inhibition by GFJ 6′,7′-dihydroxybergamottin on the first-pass elimination of midazolam and simvastatin has been predicted by PBPK simulations [151].

Animal models (e.g., normal, transgenic, or humanized animals) are widely used and may also provide valuable information regarding the potential for *in vivo* botanical-drug interactions. However, animal studies alone are not predictive of human interactions because of species differences in pharmacokinetics, sub-

strate metabolic routes and rates, proteins specificity, and inhibitor selectivity [152].

Well-designed clinical studies using specific CYP and P-gp probes [149] in healthy volunteers or patients may generate the most clinically relevant interaction data. Trials using CYP probe cocktails have been explored to efficiently quantify the potential for interactions. Several botanicals have been evaluated in this fashion, including *Echinacea* [153], saw palmetto (*Serenoa repens)* [153], SJW [45,52], kava [29], goldenseal [29], black cohosh [29], garlic [46,154], valerian [29], and ginkgo [45] (**Tables 1** and **2**).

Challenges of predicting botanical-drug interaction

Lack of standardization of the active constituents: Unlike chemically defined drugs, botanicals are mostly complex mixtures of multiple active constituents with potentially different, and often unknown, mechanisms of action and modulating effects on enzymes and transporters. An array of intrinsic (e.g., plant species, organ specificity, seasonal variation) and extrinsic (e.g., environment and cultivation methods, manufacturing processes, contamination) factors greatly define botanical products composition and quality. It has been demonstrated, for example, that the content of malonyl ginsenosides in the same sample of *Panax ginseng* roots ranged from 0% to 1.35% using different extraction methods and decreased with increasing storage time. Additionally, the total ginsenosides were underestimated and determined imprecisely by ignoring the presence of malonyl ginsenosides [155]. The concentration variability of the marker constituents (i.e., chemically based standardization) of commonly used botanicals in the US from different formulations, manufacturers, or even batch-to-batch is illustrated in **Fig. 1**. Similarly, in a study commissioned by the FDA in 1999, only 46% and 12–24% of botanical products and extracts, respectively, were consistent with the ingredients listed in the label [156].

To mitigate botanical product quality issues, as of June 2010, manufacturers and distributors in the US are required to manufacture, label and store products in compliance with good manufacturing practices [157]. One hundred percent identity testing of components is required, unless an exemption is granted based on data that showed that less than 100% identity testing will not diminish the assurance of correct components [158]. The FDA will also inspect manufacturing facilities and carefully monitor production and labeling to ensure that the product is packaged and labeled as specified in the master manufacturing record [157]. While GMP protocols will provide at least that a predetermined amount of a marker constituent is present in each botanical product from the same manufacturer, one specific product may have a different profile of constituents than another from a different source (**Fig. 1**) given the poorly standardized manufacturing process among producers. Therefore, extrapolation of scientific data or findings is difficult or even impossible.

Furthermore, the presence of specific concentrations of marker compounds may not guarantee enzyme/transporter modulation or pharmacologic activity. For example, commercially available SJW products used in most clinical studies are usually standardized to a fixed content of hypericin (**Fig. 1B**); however, hyperforin is the active constituent responsible for CYP3A4 induction [35,36]. Different commercial preparations and dry extracts of SJW showed diverse PXR-mediated induction of CYP3A4, with enzyme induction magnitude being correlated to the content of hyperforin in the extract, but not hypericin and flavonoids [159]. Moreover, SJW extracts with low hyperforin content (less than 1 mg daily) have not demonstrated any clinically relevant interactions, including with CYP3A4 and P-gp drug substrates [51,160–163]. As demonstrated in **Fig. 1B**, the measured amounts of hypericin and hyperforin varied considerably among different manufacturers.

For effective botanical-drug interaction evaluation in clinical settings, researchers must be aware of the aforementioned quality issues. Proper identification of the botanical (Latin binomial and authority) and the part(s) used in the preparation of the product including the processes used to extract and isolate the purported active constituents is the first step to ensure some minimal characterization of botanical products and define a potential botanical-drug interaction [164]. The content of the purported perpetrator(s) of interaction in the formulation and its systemic exposure should be measured whenever possible.

Extrapolation of results from in vitro studies: Prediction of the *in vivo* modulation effect of botanical products from *in vitro* data is usually problematic. Besides the multiplicity of constituents at variable concentrations as discussed above, the poor and/or variable absorption of the active inhibitor(s)/inducer(s) may lead to different *in vivo* effects. For instance, the effect of milk thistle (standardized seeds extract contains at least 30–65% flavonolignans including the active constituents silybin A and B collectively known as silymarin) on several enzymes and transporters has shown a conflicting *in vitro*/*in vivo* correlation. While a dose-dependent inhibitory effect of silymarin or silybin on various CYP isoforms, including CYP3A4 (K_i range = 5–160 µM) and CYP2C9 (K_i range = 5–19 µM) was observed in several *in vitro* studies [17, 165–167], the PK of CYP3A4 substrates nifedipine [168] and midazolam [153] were not affected by silymarin co-treatment (560 mg/day for 1 day and 175 mg of standardized extract to 80% silymarin, bid, 28 days, respectively) in healthy volunteers. On the contrary, a CYP2C9 genotype-dependent interaction between losartan (CYP2C9/CYP3A substrate) and silymarin (420 mg/day for 14 days) was demonstrated [169]. Regarding transport modulation, silymarin (50 µM) decreased [^{3}H] dehydroepiandrosterone (DHEA) uptake in OATP-B1-expressing cells [84] and increased the intracellular accumulation of [^{3}H] daunomycin (P-gp substrate) and mitoxantrone (BCRP substrate) in P-gp- [170] and BCRP- [171] overexpressing cells; although silymarin (7 µg/mL-2 ng/mL of silybin) had no effect on P-gp activity in Caco-2 cells [28]. In healthy volunteers, on the other hand, no changes in the PK of rosuvastatin (OATP1B1/BCRP substrate; not extensively metabolized) were observed with silymarin cotreatment (520 mg/day for 3 days) [172]. Likewise, silymarin did not alter the PK of digoxin [173] (900 mg/day for 14 days) and the dual CYP3A4/P-gp substrates indinavir [174–176] and ranitidine [177], while the oral clearance of metronidazole and its major metabolite was increased by 30% and C_{max} and AUC decreased by silymarin coadministration (140 mg/day for 9 days) [178]. This discrepancy may be caused by the low bioavailability (~ 1%) of the commercially available formulations of milk thistle [179, 180] used in the clinical studies. As such, sufficient systemic concentrations of the inhibitors (active flavonolignans) may not be reached. For instance, the maximum plasma concentration of total (free and conjugated) silybin achieved after ingestion of 600 mg milk thistle extract (57% silybin) was around 0.4 µM [181] which is below the lower end of the inhibition constants of CYP2C9 and CYP3A4 (Ki = 5 µM) [17, 165–167]. Additionally, the oral bioavailability of flavonolignans is highly variable [180]. Potential inter-product variation of the active constituents may also occur, which can confound the interpretation of study results.

Differential effect on intestinal and hepatic CYP3A: The potential for a differential effect of a botanical constituent on intestinal and hepatic CYP3A makes the prediction of botanical interactions with CYP3A substrates difficult. For instance, the activity of CYP3A at intestinal and hepatic sites was selectively modulated by *Echinacea purpurea* (400 mg, qid, 8 days) in healthy subjects. The intestinal availability of oral-administered midazolam was increased by 85% as result of inhibited intestinal CYP3A, but the oral clearance was not altered. Conversely, the systemic clearance of intravenous midazolam, a reflection of hepatic CYP3A activity, was increased by 34% [182]. A subsequent trial reported no effect of *E. purpurea* (1600 mg/day) on the activity of CYP3A with the probe midazolam dosed orally [153]. Therefore, the prediction of whether *Echinacea* interaction will result in inhibition or induction of CYP3A activity will be dependent on the hepatic and intestinal extraction ratios of the interacting CYP3A substrate [182]. For instance, drugs with minimal first-pass metabolism may demonstrate an increased oral clearance due to the induction of hepatic CYP3A; whereas drugs with high first-pass elimination may demonstrate increased exposure (C_{max} and/or AUC) due to the inhibition of intestinal CYP3A.

Single-dose administration vs. multiple dosing: Because of the biphasic effect of some botanicals, results from single-dose studies may be different from chronic dosing, as exemplified by several reported interactions indicating that inhibition and induction mechanisms depend on the exposure time. For example, differential SJW effects on CYPs and/or P-gp between short-time (1–3 days) versus chronic administration (> 14 days) have been observed [183]. While SJW (900 mg/day) did not alter the pharmacokinetics of the CYP3A4 probes midazolam [50] and alprazolam [184] when administered to healthy volunteers for 2 or 3 days, respectively, the oral clearance of midazolam was increased after 14 days administration (900 mg) [50,185]. Another example, a 43% reduction of voriconazole AUC (CYP2C19/CYP2C9/CYP3A4 substrate) was observed after SJW exposure (300 mg extract) for 15 days, whereas short-term dosing resulted in a 22% increase of voriconazole AUC [58]. Similar effect is observed in regard to SJW modulation of P-gp. A single dose of SJW (Sundown®, 900 mg) increased C_{max} and decreased CL/F of fexofenadine by 45% and 20%, respectively, indicating an inhibition of intestinal P-gp activity; while the long-term administration of SJW (900 mg/day, 14 days) did not alter fexofenadine disposition relative to an untreated period [72]. Conversely, in another study with the same SJW dose (Jarsin 300® 900 mg/day, 14 days) a 35% decrease in C_{max} and 47% increase in CL/F of fexofenadine was observed, possibly as a result of P-gp induction [186].

Effect of patient-intrinsic factors: Patient-related factors including genetic polymorphisms of CYPs and genes that encode transporters, such as MDR1 for P-gp, may contribute to the net outcome of a drug interaction. As such, botanical-drug interactions via metabolic or transport pathways also have the potential to be influenced by genetic factors. Genetic information has been incorporated in study designs to explore the interplay of genetic polymorphisms and combined use of botanical products. For example, the technique of gene expression profiling in rodent liver has been applied to identify the genetic mutations in metabolic pathways influenced by botanicals and facilitate more precise targeting of human studies [187]. Pharmacogenomic studies in humans may help to identify the interactions which may be more pronounced or only occur in specific groups of subjects. For example, the effects of coadministered milk thistle extract (420 mg/day of silymarin, 14 days) on the PK of losartan (CYP2C9/CYP3A substrate) and its active metabolite E-3174 were investigated in CYP2C9 genotyped healthy men. In both CYP2C9 wild-type and CYP2C9*1/*3 carriers, silymarin decreased the AUC of E-3174 while increasing the AUC of losartan. The losartan metabolic ratio was reduced in CYP2C9 wild-type subjects but not in those with CYP2C9*1/*3 genotype. Thus, silymarin inhibited the metabolism of losartan to E-3174, and the magnitude of the interaction was dependent on CYP2C9 genotype [169]. Likewise, ginkgo has demonstrated modulation of CYP2C19 expression and activity in a genotype-dependent manner [188] (**Table 2**).

Botanical Products: Regulatory Perspectives

In the United States, most botanicals are sold as dietary supplements. The 1994 Dietary Supplement Health and Education Act (DSHEA) provides regulatory framework for the safety and labeling of dietary supplements [189], defined as products taken by mouth that contain a "dietary ingredient" intended to supplement the diet. Dietary ingredients include herbs or other botanicals, vitamins, minerals, and amino acids. For a new dietary ingredient (NDI), a pre-market review of safety data is required by law [189]; otherwise, manufactures are exempted to provide the FDA with the evidence it relies on to substantiate safety or effectiveness. The majority of FDA's efforts related to dietary supplement safety are focused on the post-marketing period as post-marketing surveillance is mandatory under the 2006 Dietary Supplement and Nonsprescription Drug Consumer Protection Act [190]. By law, manufactures and distributors of dietary supplements are required to record and report to the FDA (MedWatch program-form 3500A), in a timely manner, information about serious adverse events associated with the use of their products that are reported directly to them [190]. The FDA Adverse Event Reporting program, mandatory and voluntary (by consumers and practitioners), provides initial signaling of safety issues, including the occurrence of drug interactions with botanical products. Additional clinical and scientific information are further compiled to serve as the basis of FDA risk mitigation actions, which includes education of the public and labeling language on prescription drug products [149,191].

To address practitioners and consumers concerns about the quality of dietary supplements, good manufacturing practice regulations were established in 2007 and became effective in 2010. Manufactures and distributors must comply with the Current Good Manufacturing Practices (cGMPs) in Manufacturing, Packaging, Labeling, or Holding Operations for Dietary Supplements [157] by adhering to a specific set of manufacturing processes, safety procedures, and packing and labeling standards to guarantee the identity, purity, strength, and composition of dietary supplements.

In 2004, the FDA published the guidance "Botanical Drug Products" to facilitate and encourage development of "new botanical drug products" [192]. The guidance describes the requirements of appropriateness of clinical efficacy and safety data, and of clinical pharmacology investigations including bioavailability and interactions between botanicals and commonly used drugs and/or dietary supplements. Chemistry/Manufacturing control procedures are also recommended. To the end of 2008, a total of 350 pre-investigational and investigational new drug applications of botanical drug products had been submitted to the Agency [193].

Botanical-drug interaction: labeling implications

Regulations of prescription drug labeling requires that drugs or food that interact in clinically significant ways with the product should be referenced in the "Highlights – Drug Interactions" section of its labeling [191]. Depending on the potential clinical consequences of an interaction, essential information for health care practitioners may be stated in the "Drug Interactions", "Dosage and Administration", "Contraindications", "Warnings and Precautions", and "Clinical Pharmacology" sections of the prescription drug label. Additionally, the 2012 FDA draft-guidance entitled "Guidance for industry: Drug interaction studies – study design, data analysis and implications for dosing and labeling" proposed a classification system for CYP inhibitors and inducers in the labeling, in an effort to improve the consistency of labeling language [149].

Labeling decisions for interactions with SJW or GFJ can be based on the metabolic and dispositional characteristics of the drugs being labeled without conducting actual *in vivo* studies to characterize the interaction. For example, if a drug is a substrate for either CYP3A or P-gp, or both, and modulation (induction) of these pathways may significantly decrease drug systemic exposure and effectiveness, cautions regarding the use of SJW are added to the label. Representative examples of drugs with labeling warnings about SJW use are listed in **Table 1S**. If a CYP3A substrate drug also has a low oral bioavailability because of extensive first-pass metabolism by intestinal CYP3A4, warnings regarding concomitant ingestion of grapefruit juice may be added to the label, depending on the drug's exposure-response relationship. For example, labeling for simvastatin and dasatinib carry warnings about GFJ use (**Table 1S**). Additionally, some SJW products carry warning language about potential drug interactions (**Table 2S**).

Conclusions and Future Perspectives

Drug interactions with botanicals have been increasingly reported. Timely identification of drugs that may interact with botanical's active constituents and the mechanism involved is essential for better clinical risk assessment. Researchers and manufacturers of botanical products are encouraged to fully apply the available guidelines and tools to evaluate potential botanical-drug interactions. Meaningful botanical-drug interaction monitoring should take into account inherent quality issues of botanicals, patient factors, and clinical experimental design. To better translate information into practice, regulations on prescription drug labeling content and format were set in place to highlight key drug interactions. New drugs that are CYP3A and/or P-gp substrates have higher potential for drug interactions, which may lead to labeling language regarding concomitant use with botanicals such as St. John's wort and grapefruit juice. Another risk management strategy includes mandatory reporting by manufacturers and distributors of any serious adverse events associated with the use of their botanical products. Assurance of product quality is enforced by the recent implemented cGMP regulations for dietary supplements. Strategies to strengthen knowledge and to facilitate communications among patients and clinicians about botanicals and potential interactions are also encouraged. With continued improvement in our understanding of the mechanism of drug interactions, the risks associated with such can be better predicted, evaluated and managed, in order to reduce the propensity of clinical significant adverse interactions.

Supporting information

Tables summarizing labeling examples of drug interactions involving St. John's wort or grapefruit products and of selected St. John's wort products, as well as a figure illustrating examples of efflux and uptake transporters in the gut wall, liver, and kidneys are available as Supporting Information.

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Disclaimer

The views presented in this manuscript are the author's and do not necessarily reflect those of the US Food and Drug Administration.

Conflict of Interest

The authors declared no conflict of interest.

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