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# NATURAL HEALTH PRODUCTS AND DRUG DISPOSITION*

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**Key Words** herbal, metabolism, transport, cytochrome P450

■ **Abstract** Botanicals such as herbal products (HPs) and nutraceuticals (NCs) are often regarded as low risk because of their long history of human use. Anecdotal and literature reports of adverse drug events (ADEs) and clinical studies with HPs are increasing, but many of the reports are incomplete and contradictory. These reports need to identify confounding factors and explain contradictory findings if they are to help health care professionals or patients understand what risks are involved. HPs are complex botanicals, not single-active ingredient (SAI) products. Studies can be confounded by different manufacturing processes and formulations, including cosmetics and food supplements; environment; chemotypes; misidentification or adulteration; and factors associated with the patient or user population such as use, total drug load, and genetics. Future studies need to be conducted with characterized product that includes all commercially available related products. Clinical trials should be relevant to the user population and take into account the confounding factors that may influence the interpretation of the findings.

## OVERVIEW

Plant products contain bioactive phytochemicals that are finding increasing importance in foods as NCs and in HPs as medicinal principles. HPs are a very diverse category of plant products and extracts; for example, they are known as

*Abbreviations used in text: ADE, adverse drug event; AUC, area-under-the-plasma-concentration-time curve; CAM, complementary and alternative medicine; Cmax, maximum concentration; Cmin, minimum concentration; CYP, cytochrome P450; EROD, 7-ethoxyresorufin O-deethylation; GST, glutathione S-transferase; HPs, herbal products; MIC, minimal inhibitory concentration; NCs, nutraceuticals; NHPs, natural health products; Pgp, P-glycoprotein; PK, pharmacokinetic; P450, cytochrome P450; TM, traditional medicine; SAI, single-active ingredient; SJW, St. John's wort; UGT, uridine diphosphoglucuronosyl transferase.

dietary supplements (United States), NHPs (Canada), phytomedicines (Europe), and traditional medicines (developing countries). HPs and NCs present many unique challenges that can confound pharmaceutical scientists and others alike—these are complex mixtures, not SAI products, having multiple pharmacological properties. The different regulatory processes in different jurisdictions that lead to different types of products available commercially confound the complexity. In the United States, HPs are sold as largely unregulated and untested supplements, and only structure function information but no therapeutic claims are permitted under the Dietary Supplements Health and Education Act (DSHEA) legislation of 1994. In Canada and under a somewhat similar system in Australia, NHPs are given a Natural Product Number for limited therapeutic claims for over-the-counter use based on established traditional use or supportive data and will be formally regulated with stricter controls on manufacturing and labeling. In Europe, phytomedicines are strictly regulated as drugs under the European Scientific Cooperative on Phytotherapy (ESCOP). Japan has a system in which some products are regulated as foods and others as drugs. In developing countries, the World Health Organization reports that approximately 80% of the world populations rely on TMs, mainly of herbal sources, in their primary healthcare (1). Indications for TMs in developing countries include more serious conditions (malaria, AIDS, parasitic diseases, etc.) than HPs in the developed countries, which are usually indicated as self-care products. The popularity of over-the-counter HPs, NCs, and medicinal products from plants or other natural sources has increased dramatically in developed countries and is one of the reasons for the present review. Whereas many purified botanical marker substances have been used or examined as potential pharmaceuticals, these SAIs are neither HPs nor NCs and will not be considered here.

Despite the popular believe that NPs are safe, these products are pharmacologically active and have inherent risk. Although the risk may be low in many cases where the product is used alone, of particular interest here are the many interactions that have been reported with enzymes affecting drug disposition. These include CYP 3A4 (2–8), 1A1 (9–13), 1A2 (4, 8, 12–15), 1B1 (16), 2A1, 2B (12–13), 2C (8, 13, 17–20), 2D6 (8, 17–21), 2E1 (4, 8, 21, 23–24), 3A1 (13), 3A5/7 (18–20), 4A/F (15, 25), 19 (26), P-glycoprotein (MDR1, ABCB1: 27–33), MRP1 (33), MRP2 (33), cyclooxygenase I and II (34), flavin-containing monooxygenease (35), glutathione S-transferase P1-1 (12, 14, 36), N-acetyltransferase (37), monoamine oxidase B (38), steroid X receptor (39), and uridine diphosphoglucuronosyl transferase (12, 40). This review provides a better understanding of what constitutes representative products and confounding factors affecting interpretation of these interactions.

Adverse effects, sometimes life threatening, have been associated with HPs or traditional medicines contaminated with excessive or banned pesticides, microbial contaminants, heavy metals, chemical toxins, or adulterated with orthodox drugs (1, 6, 41). Contamination may be related to the source of these herbal materials. Mycotoxins may arise during growth of fungi from unfavorable or improper storage conditions. Many examples of adulteration and substitution exist. For example, the

substitution of *Eleutherococcus senticoussus* by Chinese silk vine led to a case of neonatal androgenization (42). In recent years, a serious situation occurred with the adulteration of *Stephania tetrandra* with *Aristolochia fangchi*, which contains nephrotoxic and carcinogenic aristolochic acids.

## Natural Product Variation

Except perhaps in jurisdictions such as Europe and Japan, or more recently in Canada and Australia, HPs generally lack the stringent quality assurance and regulatory oversight of therapeutic products. Unlike SAIs, botanical raw material may be sourced from several regions or countries and may have unique genotypic and phenotypic characteristics that may confound product selection for clinical examination. Examination of one or even a few samples may inadvertently lead to testing of a single chemotype. A chemotype is a population of plants belonging to a particular species that differs chemically from others of that species, although the plants look identical. For example, Binns et al. (43) recently identified a distinct chemotype of the popular herb *Echinacea angustifolia*. The clinical effects of products containing different chemotypes (44–46) may vary.

HPs and NCs may contain constituents from many biosynthetic classes of phytochemicals (Table 1). Phytochemical diversity and redundancy can result in plant species having several different classes of phytochemicals (diversity) and multiple analogs of each biosynthetic type (redundancy). An example is valerian root, used as a mild sedative, tranquilizer, and sleep inducer (47). The major constituents include approximately 0.4%–1.4% monoterpenes; sesquiterpenes, including ß-bisabolene, caryophyllene, valerianol, valeranone, pacifigorgiol; patchouli alcohol; valerenol; valerenyl esters; valerenal; valerenic acid (with acetoxy and hydroxy derivatives); caffeic acid; gamma-aminobutyric acid; chlorogenic acid; ß-sitosterol, methyl 2-pyrrolketone; choline; hydroxypinoresinol tannins; gum; volatile oils; and resin. Shohet et al. (48) examined 31 commercial valerian preparations available in Australia and found substantial product heterogeneity of the marker phytochemicals, valerenic acid and its derivatives, ranging from $<0.01$ to 6.32 mg/g of product. Powdered capsules, on average, contained the highest concentration (2.46 mg/g) and liquids the lowest concentration (0.47 mg/ml). The mean concentration of these markers in 5 standardized products (3.56 mg/g) was significantly higher than in the 26 nonstandardized products (0.89 mg/g). Valepotriates were found at low levels ($<1$ mg/g) in some teas but were not detected in any of the finished products.

Other sources of inherent variation include environmental conditions during growth, harvest and storage conditions, and manufacturing and compounding processes. The presence of nonactive conjugates that are converted to an active moiety is another source of variation. Seasonal and environmental variation has been shown to affect the essential oil extracts from the aerial parts of *Santolina rosmarinifolia* (49) and *Hypericum perforatum* accessions grown at three experimental sites in Switzerland followed over a two-year period (1995–1996) (50).

**TABLE 1** Botanical sources of chemicals implicated in interactions

| Chemotaxonomic category | Typical interacting compounds | Botanical source |
|---|---|---|
| Monoterpenes | Limonene, sobrerol, geraniol | Green and yellow vegetables, cereals, grains, citrus peel oils, celery seed oil, herb extracts |
| Organosulfur compounds | Diallyl sulfides | Onions, garlic, leeks, shallots |
| | Isothiocyanates | Cruciferous vegetables, horseradish, radishes |
| Phenolics—polyphenols | Flavonoids (genistein, naringenin) | Green and yellow vegetables and fruits, soy products, berries, onions, garlic, citrus fruits, licorice, spices |
| | Theaflavins | Black tea leaves |
| | Catechins | Green tea leaves, berries |
| | Curcuminoids (curcumin) | Turmeric root, curry products |
| | Gingerols and diarylheptanoids | Ginger |
| | Hydroxycoumarins | Umbelliferae, horse chestnut, chamomile |
| | Furanocoumarins | Grapefruit, Earl Gray tea, rue, celery |
| | Acids (cinnamic, ellagic, sinapic) | Cinnamon, coffee beans, soybeans, grapes, strawberries, raspberries |
| | Indoles | Cruciferous vegetables |
| | Lignans (sesamol, sesaminol) | Sesame seeds and oil |
| | Chlorophyll derivatives (chlorophyllin) | All plants and vegetables |
| | Hypericin, hypericum | St. John's wort |
| Tocopherols—tocotrienols | $\alpha$-Tocopherol (vitamin E), $\alpha$-tocotrienol | Green and yellow vegetables, cereals, grains, citrus peel oils, celery seed oil, herb extracts, mint oil |
| Triterpenes | Liminoids (limonene, ichangin) | Citrus fruits |
| | Plant sterols (phytosterols) | Green and yellow vegetables, fruits, grains and cereals, soybeans |
| | Saponins/sapogenins | Ginseng (leaves, roots), soybeans |
| | Glycyrrhetic acid and derivatives | Licorice root |
| Others | Gluten, fiber | Whole grains |
| | Protease inhibitors, phytic acid | Soybeans |
| | Phthalides | Celery, parsley, carrots |

Differences were found in constituents in *H. perforatum* aerial parts (51). Some plant constituents are sugar conjugates (glycosides) that are generally unable to affect drug metabolism. Soybeans were analyzed by HPLC for the isoflavones daidzein and genistein and their respective glycoside derivatives to determine if amounts of these compounds could reliably predict the activity of the variety or year (20). Genistein ranged from 5.8 to 28.7 $\mu$g/g and daidzein from 0 to 42.6 $\mu$g/g. The glycosides daidzin and genistin were present in much larger amounts, ranging from 198 to 792 and 458 to 1261 $\mu$g/g, respectively. The free aglycones accounted for less than 7% of the total in these samples. Neither the concentration of the individual compounds nor their total correlated with inhibition of 3A4 across genotypes and years. In addition, there are individual variations in the amount taken, dosage form, preparation, length of use (first time or repeated use), combination with other products, genotype, and health status of the user.

HPs and NCs may be fresh or prepared botanicals, distillates, or extracts. For example, the processing procedures for garlic can be broadly classified into four categories: dried or dehydrated without enzyme deactivation, aqueous or oil extraction, distillation, and heating including frying and boiling (52). The products are formulated as oils of steam-distilled garlic, garlic macerated in vegetable oils, garlic powder, or gelatinous suspensions. The variation in composition as well as the instability of some constituents poses serious problems for standardization and comparison between related products.

## Labeling Information

Information printed on some labels for products such as *Echinacea*, SJW, and valerian root state that the products were standardized. SJW may be standardized to 0.3% hypericin or 4% hyperforin. Some labels mention hypericins. Wide variation in hyperforin (0.006%–2.64%), hypericin (0.008%–0.08%), and pseudohypericin (0.014%–0.19%) content was observed in tested products (52a). In these products, the 0.3% hypericin standard was only approached (0.26%) when total hypericin content was determined. Valerian root labels mention valerenic acid, valerenic acids, and valeric acid (52b). This is indicative of the confusion created by the industry, as valeric acid is a five-carbon molecule that is not related to the much larger valerenic acids. Silymarin is considered the active constituent of the milk thistle seed. It is a mixture of several flavonolignans, including silibinin (silybin A and B), isosilybinin, silichristin (silychristin), and silidianin. Constituent analysis of five milk thistle extract products identified six major constituents: 3.3% taxifolin, 23.6% silychristin, 5.3% silidianin, 20% silybin A, 30.7% silybin B, and 17.3% isosilybin (B.C. Foster, C.E. Drouin, J.F. Livesey, J.T. Arnason & E. Mills, unpublished findings). The total amounts of silybin A and B ranged from 45.7 to 61%. The biological effect of each constituent is not known. Spectrophotometric analysis as used by many producers and investigators for quality control would not provide sufficient information for a critical comparison of these products.

Another example of how labels may be misleading or confusing is the term Echinacea. *E. angustifolia* (*syn E. pallida* var. *angustifolia*) and *E. purpurea* are widely used as botanical medicines (43). A third species *E. pallida* (*E. pallida* var. *pallida*) has been widely used in Europe. A second example is Echinacea where the whole plant has been used for therapeutic purposes, but products can consist of *E. purpurea* herb extracts; combination root and herb extracts; root extracts of the three-species, single-entity, blended herbal teas with other botanicals; and blends of *E. purpurea* and *E. angustifolia* leaves, stems, and flowers plus a dry extract of *E. purpurea* roots. The constituents of Echinacea include alkamides, caffeic acid derivatives, glycoproteins/polysaccharides, and ketoalkenynes (43, 53–54), but label information indicated that standardization is focused on phenols (4%) or 4% echinacoside. Echinacoside is only a marker for *E. angustifolia*. Echinacoside and cichoric acid (Table 2) were not detected in some products (R.K. Drobitch, A. Krantis, M. Panahi, J.T. Arnason, K. Kramp, F.J. Burczynski, C. Briggs, P. Jiang & B.C. Foster, unpublished data). All extracts markedly inhibited CYP-mediated metabolism (Table 2). The findings with soft gel products were the most variable. Aliquots of the four soft gel products had moderate to high activity toward CYP2D6 and 3A4, but only NRP 69 and 72 had an inhibitory effect against CYP2C9. In addition, NRP 71 did not inhibit CYP2C19-mediated metabolism.

## Confounding Factors in Experimental Evaluations

Small changes in the lipophilic (or polar) nature of the extraction solvents used in assays can greatly alter the results of the assays. A garlic product was extracted with a sequential series of solvents ranging in lipophilicity from hexane (yellow-green extract) followed by chloroform (brown-green), ethyl acetate (bright red), methanol (orange-red), 55% ethanol (light peach color), and finally water (very faint peach color) (18). Results suggesting the presence of fluorescent substances were observed when testing the aliquots of ethyl acetate (169.9%) and hexane (157.0%) extracts against 3A4. The chloroform and methanol extracts also had high inhibition with values of 97.6% and 87.5%, respectively, but the weaker solvents in this sequential extraction protocol, 55% ethanol and water, were less inhibitory (20.6% and 6.3%, respectively). A series of nonsequential extracts also gave high activity in all extracts. As differences in the inhibitory effect of aqueous and methanolic extracts of fresh and aged garlic cloves on 3A4-mediated metabolism were noted previously, the three varieties were extracted under four different conditions. Results varied with variety, but in general, distilled water and phosphate buffer extracts gave the strongest overall suppression effect in isoform-mediated metabolism of marker substrates.

Intrinsic (natural) fluorescence and quenching are confounding variables in fluorescence-based enzyme inhibition assays of natural products. Zou et al. (55) measured the fluorescence and quenching properties of 25 components of popular herbal products. These analyses were performed under conditions typically

**TABLE 2** Biomarker analysis and percentage inhibition of human cytochrome P450-mediated metabolism by aliquots of 1.25 mg/ml extracts from products containing *Echinacea* ($n \geq 6 \pm SD$)

| | Form | Cichoric acid | Echinacoside | 2C9 | 2C19 | 2D6 | 3A4 |
|---|---|---|---|---|---|---|---|
| 10B | Tea | 2572 | 953 | 72.6 ± 5.86 | 73.4 ± 3.13 | 91.4 ± 1.17 | 64.5 ± 0.38[c] |
| 27 | Tea | 5895 | ND | 52.4 ± 6.42 | 77.1 ± 7.35 | 80.0 ± 0.61 | 79.9 ± 0.49 |
| 62 | Tea | 8916 | 258 | 65.0 ± 2.01 | 61.2 ± 6.13 | 66.8 ± 1.46 | 66.1 ± 0.73 |
| 73[a] | Tablet | 1852 | 32,423 | 71.9 ± 7.07[d] | 85.3 ± 4.93[d] | 65.5 ± 1.67 | 41.5 ± 4.03 |
| 74 | Tablet | ND[b] | ND | ND | 5.9 ± 10.19[d] | 16.2 ± 2.03 | 9.3 ± 3.52 |
| 29 | Tincture | ND | ND | 59.0 ± 3.45 | 54.9 ± 4.54 | 60.7 ± 1.23 | 48.1 ± 1.49 |
| 69 | Softgel | 1920 | ND | 52.5 ± 7.73 | 49.4 ± 6.88 | 49.8 ± 0.75 | 68.5 ± 0.75 |
| 71[a] | Softgel | 2299 | 793 | ND | ND | 98.2 ± 0.55 | 91.4 ± 2.22 |

[a]Standardized to 4% echinacosides.

[b]Not detected.

[c]652 $\mu$g/ml stock solution.

[d]5 mg/ml stock solution.

employed in drug-drug interaction studies using c-DNA-derived P450 isoforms and surrogate fluorogenic substrates. Four of the 25 compounds tested (isorhamnetin, quercetin, vitexin, and yangonin) fluoresced or quenched sufficiently to interfere with these assays. Intrinsic fluorescence had a greater effect on these assays than quenching, and for one compound, yangonin, it was sufficient to mask inhibition and potentially produce a false negative result. Quenching was sufficient with quercetin, to mimic "weak" inhibition. The intrinsic fluorescence or quenching capacity of HPs may confound certain fluorometric assays, making it imperative that proper controls are included in the evaluation studies on these products. The degree of interference may preclude the assay or require supporting evidence from chromatographic assays where this factor can be separated from the test substrate and metabolite.

Dissolution of constituents from teas is an important consideration (56–58). Dissolution rates are routinely performed with synthetic drugs; however, with HPs this crucial property is often not investigated. Using procedures of the European Pharmacopoeia, Taglioli et al. (58) evaluated the dissolution behavior of capsules containing various herbal drugs (Passira, Senna, Ginkgo) manufactured by different methods. Active components or marker constituents were analyzed. Adequate dissolution behaviors of the flavonoids of Ginkgo were obtained for all preparations, whereas for Passiflora and Senna only the extracts showed a complete dissolution of the marker flavones and sennosides, respectively. Three different patterns were noted when four HPs were examined using tea bag infusions (20). The initial 10-min values for Echinacea Special tea were higher than the other NHPs, but the inhibition curve only increased slightly with time. Goldenseal herb, Echinacea and Goldenseal had low initial values that nearly doubled after a second 10-min incubation. The third pattern with Feverfew showed a linear increase in marker extraction through the incubation period. Visual examination of these products found inter- and intraproduct differences in particulate size, ranging from fine powder to substantially intact leaves and stems. As product dissolution varies between products, it is expected that there will be clinical differences based on the amount and temperature of the water, extent of agitation, and time the teas are allowed to brew.

## Stability

Bilia et al. (59–60) investigated the stability of 40% and 60% v/v tinctures of artichoke, SJW, Calendula flower, Milk thistle fruit, and Passionflower. Stability was related both to the class of flavonoids and water content of the tinctures. Shelf life at 25°C of the most stable tincture (Passionflower 60% v/v) was approximately six months, whereas that of the Milk thistle tinctures was approximately three months. Budzinski et al. (61) examined 21 tinctures and noted that there was marked variation in the ability of these products to inhibit CYP3A4-mediated metabolism. Several clinical trials have subsequently been conducted on some of the products examined in this study. Based on the stability concerns (59–60)

it would be interesting to revisit this area to examine freshly manufactured and expired commercial products.

Thermal and photostability of a commercial dried extract and capsules of SJW were also evaluated (59). Photostability testing showed all the constituents to be photosensitive in the tested conditions. However, different opacity agents and pigments influenced stability. Amber containers had little effect on the photostability of the investigated constituents. Assays should be performed under reduced or F40 gold fluorescent lighting to minimize potential for photodecomposition or activation (18). Long-term thermal stability testing showed a shelf life of less than four months for hyperforins and hypericins, even when ascorbic and citric acids were added to the formulation.



## SELECTED EXAMPLES

### Citrus

The potential for some members of the Rutaceae family, such as grapefruit, lime, and Seville orange, to interact with 3A4 and Pgp and affect PK has been extensively examined (2, 4–8, 10, 62–67). The mechanisms involved are understood in part. Activity was initially attributed to bioflavonoids and then to furanocoumarins. Furanocoumarin derivatives can be characterized into two main groups: angular with a furan ring attached to 7,8-position or linear with the furan ring at 6,7-position with methoxy, prenyloxy, and geranlyoxy substitution at 5- and/or 8-position. Some, but not all are inhibitory (10). In grapefruit, geranyloxy derivatives of furanocoumarins (psoralens) are thought to be involved in competitive or mechanism-based inhibition (10).

What is generally poorly understood is that furanocoumarins, natural light-activated toxins that protect plants from herbivores such as insects and microbes, are also present in plants belonging to the Fabaceae (legumes), Moraceae (fig and mulberry), and Apiaceae (carrot, celery) families (10). Hot water decoctions or 40% ethanol infusions from Apiaceae: Baizhi (*Angelica dahurica* and varieties), Qianghuo (*Notopterygium incisum* or *N. forbesii*), Duhuo (*Angelica biserrata*), Fangfeng (*Saposhnikovia divaricata*), Danggui (*Angelica sinensis*), and Rutaceae: Zhishi or Zhiqiao (*Citrus aurantium*) resulted in various degrees of human CYP3A inhibition as determined by microsomal testosterone 6ß-hydroxylation (67). The inhibitory potency was consistent with the abundance of the hydrophobic components for each sample. Some products showed increased inhibition after preincubation, suggesting mechanism-based inhibition. Some formulated prescriptions, however, showed intense inhibition with their hydrophilic fractions rather than with their hydrophobic fractions, suggesting that components other than furanocoumarins in herbal prescriptions may also cause CYP3A inhibition. Studies suggest that furanocoumarins can inhibit or induce a wide range of P450s in addition to CYP3A4, such as CYP1a1, CYP1A2, Cyp1b1, Cyp2a5, CYP2A6, CYP2B1, CYP6B1/3, CYP6B4, and CYP6D1 (10).

## Cranberry Juice

Cranberry juice (*Vaccinium macrocarpon*) is a popular drink that has been used to reduce or prevent urinary infections. Five reports suggesting changes in the International Normalized Ratio (INR) values for the Prothrombin Time have been received by the Committee on Safety of Medicines, indicating an interaction between cranberry juice and warfarin, including one fatal case (68).

## Garlic

Garlic (*Allium sativum* L.) and garlic products generally have been regarded as safe, but conflicting reports in the literature make it difficult to unequivocally establish the clinical efficacy and safety of these products either alone or in the presence of therapeutic products. One case report identified two HIV-infected persons taking garlic or garlic supplements for more than two weeks who developed severe gastrointestinal toxicity after beginning ritonavir-containing antiretroviral therapy (400 or 600 mg twice daily) (69). The symptoms, including nausea, vomiting, and diarrhea, resolved with discontinuation of garlic or ritonavir. A recent study analyzed 24 representative garlic products, including three fresh garlic bulbs (P.S. Ruddock, M. Liao, B.C. Foster, L. Lawson, J.T. Arnason & J.R. Dillon, unpublished data). Interestingly, within the odorless garlic entities, the range for the allicin/alliin ratio varied from 0 to 4.7. The major biomarker varied with manufactured product, and the constituent content did not correlate with the in vitro inhibition of CYP-mediated metabolism. The results were consistent with earlier findings on their inhibitory effect on CYP 2C9*1, 2C9*2, 2C19, 2D6, and 3A-mediated metabolism (45). Extracts from garlic exhibited a similar inhibitory effect on all 3A isoforms. Chinese and elephant garlic (*Allium ampeloprasum*) had a lesser inhibitory effect on 3A7; Chinese garlic extracts also had a lesser effect on the 3A5 isoform studied. All fresh varieties had a slight inhibitory effect on 2C9*1-mediated metabolism but highly stimulated metabolism of the marker substrate with the 2C9*2 isoform. The extracts had negligible to no effect on 2C19- and 2D6-mediated metabolism. However, all extracts strongly inhibited 3A4-mediated metabolism. The effects of aqueous extracts from aged garlic capsules and the three fresh varieties were examined for their ability to interact with human Pgp. Relative to 20 $\mu$M verapamil as the positive control, the phosphate buffer extracts of aged, common, and Chinese garlic had moderate levels of product-stimulated vanadate-sensitive ATPase activity. Elephant garlic was inactive.

The effect of odorless garlic on single-dose pharmacokinetics of ritonavir was examined in ten healthy volunteers (five male, five female) who received 400 mg of a single dose of ritonavir either alone or with 10 mg of odorless garlic in a randomized crossover design (70, 71). Coadministration of garlic decreased the AUC by 17% (90% CI, −31% to 0%; range −46% to 68%) and decreased peak plasma concentration of ritonavir by 1% (90% CI, −25% to 31%; range −51% to 136%). Although the trend was toward lower levels of ritonavir, the effect was not significant. In a longer study, 10 healthy volunteers received 10 doses of saquinavir at a dosage of 1200 mg 3 times daily with meals for 4 days on study days 1–4,

22–25, and 36–39, and they received a total of 41 doses of garlic caplets taken 2 times daily on study days 5–25 (72). In the presence of garlic, the mean saquinavir AUC during the 8-h dosing interval decreased by 51%, trough levels at 8 h after dosing decreased by 49%, and the Cmax decreased by 54%. After the 10-day washout period, the AUC, trough, and Cmax values returned to 60%–70% of their values at baseline. Markowitz et al. (73) reported contradictory findings with no effect on 2D6-mediated metabolism of dextromethorphan and 3A4-mediated metabolism of alprazolam with no significant differences in pharmacokinetic parameters at baseline and after garlic extract treatment.

Foster et al. (74) demonstrated that garlic and SJW could have an antagonistic or synergistic effect on antibiotics, indicating that herbal effects on host drug disposition mechanisms may also affect response to antibiotics. Ward et al. (75), using *Staphylococcus aureus* ATCC 29,213 or *Escherichia coli* ATCC 25,922 as the indicator organisms, showed a general increase in the MIC of ampicillin by the products they studied. There were 13 product-related increases in the MIC and 2 decreases. All garlic products increased the MIC of norfloxacin-sensitive organism to greater than fourfold above baseline. With *Escherichia coli* ATCC 25922, the greatest product-antibiotic interaction was with the ampicillin-sensitive organism. Garlic, Echinacea, and zinc products all caused large increases in the MIC to ampicillin over baseline values.

## *Ginkgo biloba*

The effects of *Ginkgo biloba* leaf extract on the pharmacokinetics of diltiazem were examined in rats (76). The simultaneous addition of extract to small intestine and liver microsomes inhibited the formation of the active N-demethyl metabolite by CYP3A in a concentration-dependent manner with an IC (50) of approximately 50 and 182 $\mu$g/ml, respectively. After a single oral pretreatment with extract (20 mg/kg), both the rate of formation of the metabolite and total amount of CYP in intestinal or hepatic microsomes decreased transiently. Pretreatment significantly decreased the terminal elimination rate constant and increased the mean residence time after intravenous administration of diltiazem (3 mg/kg). Furthermore, it significantly increased the AUC and absolute bioavailability after oral administration of 30 mg/kg. These results indicated that the concomitant use of *Ginkgo* extract in rats increased the bioavailability of diltiazem by inhibiting both intestinal and hepatic metabolism, at least in part, via a mechanism-based inhibition for CYP3A.

A study in healthy volunteers phenotyped as CYP2D6 extensive metabolizers with *Ginkgo biloba* (77) concluded that the products used in these studies at the recommended dose was unlikely to significantly alter the disposition of coadministered medications primarily dependent on the CYP2D6 or CYP3A4 pathways for elimination.

## Goldenseal

Goldenseal (*Hydrastis canadensis* Ranunculaceae), a popular herbal supplement for gastrointestinal ailments, has been shown to affect CYP3A4-mediated

metabolism and ATPase activity (27, 61). It contains the alkaloids berberine and hydrastine, hydrastinine, and canadine. Extracts of goldenseal containing approximately equal concentrations (approximately 17 mM) of two methylenedioxyphenyl alkaloids, berberine and hydrastine, inhibited with increasing potency (CYP2C9) diclofenac 4′-hydroxylation, (CYP2D6) bufuralol 1′-hydroxylation, and (CYP3A4) testosterone 6ß-hydroxylation activities in human hepatic microsomes (17). The inhibition of testosterone 6ß-hydroxylation activity was noncompetitive, with an apparent Ki of 0.11% extract. Of the methylenedioxyphenyl alkaloids, berberine (IC50 = 45 $\mu$M) was a better inhibitor toward bufuralol 1′-hydroxylation and hydrastine (IC50 approximately 350 $\mu$M for both isomers), and was an inhibitor toward diclofenac 4′-hydroxylation. For testosterone 6ß-hydroxylation, berberine was the least inhibitory component (IC50 approximately 400 $\mu$M). Hydrastine inhibited testosterone 6ß-hydroxylation with IC50 values for the (+)- and (−)-isomers of 25 and 30 $\mu$M, respectively. For (−)-hydrastine, an apparent Ki value of 18 $\mu$M without preincubation and an NADPH-dependent mechanism-based inhibition with a $k_{inactivation}$ of 0.23 $min^{-1}$ and a KI of approximately 110 $\mu$M were determined. CYP metabolic-intermediate complex formation could be demonstrated for both hydrastine isomers. Hydrastine formed a CYP complex with CYP2C9, CYP2D6, and CYP3A4. Coexpression of cytochrome b5 with the CYP isoforms enhanced the rate but not the extent of complex formation.

The pharmacokinetics of indinavir in 10 healthy volunteers before and after 14 days of treatment with goldenseal root (1140 mg twice daily) were not altered significantly (78).

Three other herbals, barberry (*Berberris vulgaris*), Oregon grape (*Mahonia aquifolium*), and Goldenthread (*Coptis groenlandica*), also contain measurable amounts of these compounds (Table 3) and have a long ethnobotanical record in

**TABLE 3** The chemical characterization and percentage inhibition of four berberine-containing botanicals on cytochrome P450-mediated metabolism of three isozymes

| Product | BER[a] $\mu$g/ml | HS[b] $\mu$g/ml | HSN[c] $\mu$g/ml | CDN[d] $\mu$g/ml | CYP 3A4 | CYP 2C19 | CYP 19 |
|---|---|---|---|---|---|---|---|
| *Mahonia aquifolium* | 124 | 5 | ND[e] | ND | 27.0 | 13.6 | 31.7 |
| *Coptis trifolia* var *groenlandica* | ND | 135 | ND | ND | 46.2 | 24.7 | 41.3 |
| *Hydrastis canadensis* | 9000 | 4801 | 209 | 46.3 | 54.0 | 49.4 | 60.1 |
| *Berberris vulgaris* | 790 | ND | ND | ND | 47.5 | 25.4 | 45.5 |
| ketoconazole | | | | | 84.2 | 40.6 | 33.9 |

[a]Berberine.

[b]Hydrastine.

[c]Hydrastinine.

[d]Canadine.

[e]Not detected.

North America (R. Leduc, I. Scott, R. Marles, J. Dillon, J.T. Arnason & B.C. Foster, unpublished findings). The relative amounts of alkaloids varied greatly in the four species tested, with *H. canadensis* having considerably higher concentrations of the marker compounds. Extracts of *H. canadensis* were inhibitory to CYP 3A4, 2C19 and 2C19. *C. groenlandica* and *B. vulgaris* were less active, whereas *M. aquifolium* extracts were the least inhibitory. These botanicals have the potential to affect human drug and intermediary metabolism independent of the concentration of the major biomarkers for these botanicals.

## Herbal Teas

Herbal and black teas were analyzed for their capacity to inhibit in vitro metabolism of drug marker substrates by human CYP isoforms (20). Aliquots and infusions of all products inhibited 3A4 metabolism. Of the aliquots from teas tested with 2C9, 2C19, and 2D6, many demonstrated inhibitory activity. Black teas and herbal tea mixtures were generally more inhibitory than single-entity herbal teas. Maliakal & Wanwimolruk (41) investigated the effect of herbal teas (peppermint, chamomile, and dandelion) on the activity of hepatic Phase I and II metabolizing enzymes using female rat liver microsomes. After four weeks of pretreatment, CYP isoforms and Phase II enzyme activities were determined by incubation of liver microsomes or cytosol with appropriate substrates. Activity of CYP1A2 in the liver microsomes of rats receiving dandelion, peppermint, or chamomile tea was significantly decreased ($P < 0.05$) to 15%, 24%, and 39% of the control value, respectively. CYP1A2 activity was significantly increased by pretreatment with caffeine solution. No alterations were observed in the activities of CYP2D and CYP3A in any group of pretreated rats. Activity of CYP2E in rats receiving dandelion or peppermint tea was significantly lower than in the control group, 48% and 60% of the control, respectively. There was a dramatic increase (244% of control) in the activity of UGT in the dandelion tea–pretreated group. There was no change in the activity of GST. The results suggested that certain herbal teas can cause modulation of Phase I and II drug metabolizing enzymes.

## Kava

Inhibition of CYP enzymes by kava extract was investigated (15, 79). Whole kava extract (normalized to 100 $\mu$M total kavalactones) caused concentration-dependent decreases in P450 activities, with significant inhibition of the activities of CYP1A2 (56% inhibition), 2C9 (92%), 2C19 (86%), 2D6 (73%), 3A4 (78%), and 4A9/11 (65%) following preincubation for 15 min; CYP2A6, 2C8, and 2E1 activities were unaffected. These data indicate that kava has a high potential for causing drug interactions through inhibition of P450 enzymes.

## Milk Thistle

Venkataramanan et al. (40) evaluated the effect of silymarin on the activity of hepatic drug-metabolizing enzymes in human hepatocyte cultures. Treatment with

silymarin significantly reduced the activity of CYP3A4 enzyme (by 50% and 100%, respectively) as determined by the formation of 6-$\beta$-hydroxy testosterone and the activity of UGT1A6/9 by 65% and 100%, respectively, as measured by the formation of 4-methylumbelliferone glucuronide. Silymarin also significantly decreased mitochondrial respiration in human hepatocytes.

At least three studies have been conducted with products containing milk thistle (*Silybum marianum*) extract to characterize the pharmacokinetics of indinavir in healthy subjects. Piscitelli et al. (80) and DiCenzo et al. (81) concluded that these silymarin products had no apparent effect on indinavir plasma concentrations. In a third study with 16 subjects lasting 28 days by Mills et al. (83), the $AUC_{0\text{-}8}$ indinavir was reduced by a mean 4.4% (90% CI, −26% to 27.5%, $P = 0.6$) from Phase I to Phase II in the active group, rebounding to a Phase III reduction of 17.3% (90% CI, −9% to 37.3%, $P = 0.6$) of baseline. Control $AUC_{0\text{-}8}$ reduced by 21.5% (90% CI, −8 to 43%, $P = 0.2$) from Phase I to Phase II and rebounded to a further reduction at Phase III of 38.5% (90% CI, 15.3% to 55.3%, $P < 0.01$) of baseline. This study has important implications for the conduct and design of herb-drug interaction trials. The significant decline of $AUC_{0\text{-}8}$ in the control group indicates that factors other than the exposure of interest may affect drug metabolism.

## St. John's wort

The in vitro and clinical effects of SJW on drug disposition and safety have also been extensively examined (3–8, 21, 31, 35, 56–57, 59, 61, 83–95). Additional studies have shown that SJW can affect CYP-mediated metabolism, transport, cell viability, and modulate induction of nitric oxide. Ruschitzka et al. (87) clearly established that concomitant use of SJW with cyclosporin could cause serious ADEs. Piscitelli (88–89) showed that SJW reduced the AUC of indinavir by a mean of 57% and decreased the extrapolated 8-h indinavir trough by 81% in healthy volunteers. This could lead to drug resistance and treatment failure. As with other HPs, there have been supportive (88, 91, 93, 94) and contradictory (90, 92) reports.

Markowitz et al. (94) assessed the potential of SJW with 12 healthy extensive metabolizers of CYP 2D6 in a 14-day study. A twofold decrease in AUC for alprazolam plasma concentration versus time ($P < 0.001$) and a twofold increase in alprazolam clearance ($P < 0.001$) were observed following SJW administration. Alprazolam elimination half-life was shortened from a mean of 12.4 h to 6.0 h ($P < 0.001$). The mean urinary ratio of dextromethorphan to its metabolite was 0.006 at baseline and 0.014 after SJW administration ($P = 0.26$).

The effect of SJW on P-glycoprotein activity was examined with use of fexofenadine as selective probe drug (93). A single dose of SJW significantly ($P < 0.05$) increased the maximum plasma concentration of fexofenadine by 45% and significantly ($P < 0.05$) decreased the oral clearance by 20%, with no change in half-life or renal clearance. Fourteen-day administration of SJW did not cause a significant change in fexofenadine disposition relative to the untreated phase. Compared with

the single-dose treatment phase, SJW caused a significant 35% decrease ($P < 0.05$) in maximum plasma concentration and a significant 47% increase ($P < 0.05$) in fexofenadine oral clearance.

The effect of SJW on CYP activity was examined with a probe drug cocktail (91). Twelve healthy subjects (five female, seven male) completed this three-period, open-label, fixed-schedule study. Tolbutamide (CYP2C9), caffeine (CYP1A2), dextromethorphan (CYP2D6), oral midazolam (intestinal wall and hepatic CYP3A), and intravenous midazolam (hepatic CYP3A) were administered before short-term SJW dosing (900 mg), and after two weeks of intake (300 mg tid) to determine CYP activities. Short-term administration of SJW had no effect on CYP activities. Fourteen-day administration caused a significant ($P < .05$) increase in oral clearance of midazolam from 121.8 ∀ 70.7 to 254.5 ∀ 127.8 and a corresponding significant decline in oral bioavailability from 0.28 ∀ 0.15 to 0.17 ∀ 0.06. In contrast to the >50% decrease in the AUC when midazolam was administered orally, 14-day administration caused a 20% decrease in AUC when midazolam was given intravenously. Fourteen-day SJW administration resulted in a significant and selective induction of CYP3A activity in the intestinal wall. SJW did not alter the CYP2C9, CYP1A2, or CYP2D6 activities in these healthy subjects.

## Spices

Ground fancy clove (Sri Lanka), ground ginger (China or India), oregano leaf (Turkey), ground sage (Turkey), thyme leaf (Spain), and ground turmeric (India) extracts were found to inhibit CYP2C9, CYP2C19, CYP2D6, and CYP3A4-mediated metabolism (20).

## Traditional Medicine Plants

Deferme et al. (29) examined extracts of 43 Tanzanian medicinal plants for their potential inhibitory effect on Pgp using the secretory transport of cyclosporin in the Caco-2 system as a measure of the functionality of Pgp efflux. Extracts of *Annickia kummeriae* and *Acacia nilotica* had a significant effect. In the presence of the extract of *A. kummeriae*, a concentration-dependent decrease in transport of cyclosporin was observed that was comparable to that of valspodar, a known Pgp inhibitor.

Traditional Chinese medicine includes both crude Chinese medicinal materials (plants, animal parts, and minerals) and Chinese proprietary medicine. They are believed by many to be safe and are used for self-medication. Although the risk appears to be low, certain products have been associated with a number of serious ADEs. A study with 12 traditional products, including one subsequently shown to contain three proprietary drugs, found most aqueous extracts inhibited CYP450-mediated metabolism of at least three isozymes (19). All liquid samples markedly inhibited the metabolism of 2C9, 2C19, 2D6, and 3A4. De le ke chuan kang and Rensheng dao were the strongest CYP inhibitors.

Ueng et al. (12) examined the effects of methanol and aqueous extracts of *Evodia rutaecarpa* on CYP, UGT, and GST in C57BL/6J mice. Methanol extract caused a dose-dependent increase of liver microsomal EROD activity. In liver, methanol extract increased benzo(a)pyrene hydroxylation, 7-methoxyresorufin O-demethylation (MROD), 7-ethoxycoumarin O-deethylation (ECOD), benzphetamine N-demethylation, and N-nitrosodimethylamine N-demethylation activities. Aqueous extract increased EROD, O-demethylation, and O-deethylation activities 68%, twofold, and 83%, respectively. For conjugation activities, methanol extract elevated UGT and GST activities. Aqueous extract elevated UGT activity without affecting GST activity. Immunoblot analyses showed that methanol extract increased the levels of CYP1A1, CYP1A2, CYP2B-, and GSTYb-immunoreactive proteins. Aqueous extract increased CYP1A2 protein level. In kidney, neither extract had any effect on most activities. Rutaecarpine, evodiamine, and dehydroevodiamine contributed, at least in part, to the increase of hepatic EROD activity.

Ge-gen, the root of a wild leguminous creeper, *Pueraria lobata* (Willd.) Ohwi (13), possesses a high content of flavonoid derivatives, the most abundant of which is puerarin. Puerarin and Ge-gen crude extracts inhibited the steady-state chemiluminescent reaction in a dose-dependent fashion. Although both CYP content and NADPH-(CYP)-c-reductase activity were significantly increased in all situations, a complex pattern of CYP modulation was observed, including both induction (puerarin: CYP2A1, 1A1/2, 3A1, 2C11; Ge-gen: CYP1A2, 3A1, 2B1) and inactivation (Ge-gen and puerarin: CYP3A, 2E1, 2B1). The latter are due to either parental agents or metabolites, as demonstrated by in vitro studies. Ge-gen contains compounds with potent antioxidant activity, which impair CYP-catalyzed drug metabolism.

Ohnishi et al. (96) examined the possibility of pharmacokinetic interactions between Sho-saiko-to extract powder, a widely used traditional Japanese herbal (Kampo) medicine and carbamazepine in rats. Sho-saiko-to inhibited hepatic microsome 10,11-epoxylase activity in a concentration-dependent manner. Liver weight, amounts of CYP and cytochrome b(5) in hepatic microsomes, and the formation of the 10,11-epoxide by microsomes were not influenced by two-week repeated oral pretreatment, although pretreatment with phenobarbital (80 mg/kg/d, i.p.) significantly increased these parameters. Simultaneous oral administration of Sho-saiko-to significantly decreased Cmax of carbamazepine and the AUC of the epoxide and lengthened the time to reach Cmax. Two-week repeated oral pretreatment with Sho-saiko-to, however, did not affect the plasma concentration-time profile or any pharmacokinetic parameter of carbamazepine. A single oral administration of Sho-saiko-to (1 g/kg) significantly delayed gastric emptying and simultaneous oral administration of TJ-9 with carbamazepine CBZ to rats decreased the gastrointestinal absorption of carbamazepine, at least in part, by delaying gastric emptying without affecting the metabolism.

Ueng et al. (97) examined the effects of Wu-chu-yu-tang on hepatic and renal CYP, UGT, and GST in C57BL/6J mice. Treatment of mice with 5 g/kg per day Wu-chu-yu-tang for 3 days caused 2.5-fold and 2.9-fold increases of liver microsomal

EROD and 7-methoxyresorufin O-demethylation activities, respectively. CYP activities toward 7-ethoxycoumarin, benzphetamine, N-nitrosodimethylamine, erythromycin, and nifedipine, and conjugation activities of UGT and GST were not affected. In kidney, Wu-chu-yu-tang treatment had no effects on CYP, UGT, and GST activities. Among the four component herbs of Wu-chu-yu-tang, only *Evodiae fructus* (Wu-chu-yu) extract increased EROD activity and CYP1A2 protein level. The main active alkaloids in *E. fructus* are rutaecarpine, evodiamine, and dehydroevodiamine. At doses corresponding to their contents in Wu-chu-yu-tang, rutaecarpine treatment increased hepatic EROD activity, whereas evodiamine and dehydroevodiamine had no effects. These results demonstrate that ingestion of Wu-chu-yu-tang increases mouse hepatic Cyp1a2 activity and protein level.



## PRODUCT SELECTION FOR CLINICAL STUDIES

The number of botanical varieties, dosage forms, and formulations in combination with variability in botanical material make it impossible to evaluate all of these products in animal models or clinical trials. How should a product be selected? Should one choose an average or superior product, as the results of the study will subsequently be viewed as representative of all related products? As an example, four SJW products with similar inhibitory activity against CYP-mediated metabolism were evaluated further for their effects on cell viability, potential to modulate induction of nitric oxide, and 1A1/2-mediated EROD activity in glial cell cultures (86). SJW A failed to induce EROD activity or nitric oxide over the concentration range studied. SJW B and C produced the highest nitric oxide levels, which could be cause for concern for CNS toxicity. SJW C produced the highest levels of resorufin, whereas SJW B and D showed minor induction of EROD activity. SJW A and D both produced significant cell toxicity as measured by LDH-release. Which product should be studied? As a minimum, several products used by the patient community should be obtained and authenticated. The selection criteria should include multiple lot testing, cost, product availability, and chromatographic separation and quantification of representative biomarker constituents and bioassay testing for relevant activities. Once a product is selected it needs to be thoroughly characterized so that future commercial products can be manufactured in a comparable way.

## SUMMARY AND FUTURE PERSPECTIVES

Botanicals such as HPs and NCs are often regarded as low risk because of the long history of human use, their natural origin, or simply because the concentration of active principles is lower than conventional drugs. All products have risk when combined with other products, even those that when used traditionally may be considered safe. There is a tendency to relate the pharmacological activity of

an HP to a SAI. In the same fashion, there is a tendency to relate the effect of a SAI on drug disposition parameters to the combined total HP, even when the SAI may only account for a small fraction of the total weight. HPs are complex products where synergistic pharmacokinetic and/or pharmacodynamic interactions are of vital importance (98, 99). Many processes, from absorption, metabolism, distribution, excretion and receptor binding may be affected. The purported pharmacological effect of the NHP must be separated from the potential effect on drug disposition, particularly if it is a negative finding from an in vitro assay.

Anecdotal and literature reports of ADEs and clinical studies with HPs are increasing. Many of these reports are incomplete and contradictory. These reports need to be standardized for clarity to appreciate the confounding factors and in some cases contradictory findings. Average PK data obtained from clinical trials in healthy subjects, with stringent exclusion criteria or when subjects with potentially confounding polymorphisms have been excluded, may not show what is relevant in the patient, regardless of what occurred in the healthy test subject. Studies with HPs can be confounded by products from different manufacturing processes and formulations, presence of these HPs in other products including cosmetics and food supplements, total drug (and xenobiotic) load (100), environmental effects on the plant, chemotypes, misidentification or adulteration of products, and factors associated with the patient or user population. When a HP has reported ADEs, demonstrated in vitro or has the clinical potential to affect drug disposition, the principal of caution should guide further use. Studies that attempt to extrapolate negative findings with HPs, particularly with SAIs, are meaningless if the confounding factors are not taken into consideration. If there is wide variance in PK ranges, this may suggest that some individuals would be at risk, particularly when product is being used off label or in subjects who would not meet the inclusion criteria of purportedly definitive studies. Contradictory findings need to be explained if they are to help regulatory agencies, health care professionals, or the patient understand what risks are involved.

Future clinical studies need to be conducted with a fully characterized product that includes comparisons to a number of commercially available related products. Clinical trials should identify a representative product and take into account the confounding factors which may influence the interpretation of the findings and be consistent with how the product is used—in some cases a 14–21 day study may be insufficient. In addition to PK information on the drug, the PK of the main markers should also be examined. In vitro studies should evaluate the effects of different solvent extracts on drug-metabolizing enzymes beyond the major human CYP 1–3 isoforms to examine other Phase I enzymes, including Phase II drug metabolism enzymes and transporters. Wider CYP screening with isoforms such as CYP 4 and 19 is required to determine if other crucial endogenous pathways are also affected.

All products have risk, with risk generally increasing in patients who have confounding health, genetic, and environmental factors, including polypharmacy. Health care professionals and their patients need relevant information on both the

benefits and the limitations of in vitro and clinical PK studies to determine what risk, if any, may be associated with their combined drug and HP exposure.

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