In vivo assessment of botanical supplementation on human cytochrome P450 phenotypes: *Citrus aurantium*, *Echinacea purpurea*, milk thistle, and saw palmetto

Objectives: **Phytochemical-mediated modulation of cytochrome P450 (CYP) activity may underlie many herb-drug interactions. Single–time point phenotypic metabolic ratios were used to determine whether long-term supplementation of *Citrus aurantium*, *Echinacea purpurea*, milk thistle (*Silybum marianum*), or saw palmetto (*Serenoa repens*) extracts affected CYP1A2, CYP2D6, CYP2E1, or CYP3A4 activity.**

Methods: **Twelve healthy volunteers (6 women, 6 men) were randomly assigned to receive *C aurantium*, *E purpurea*, milk thistle, or saw palmetto for 28 days. For each subject, a 30-day washout period was interposed between each supplementation phase. Probe drug cocktails of midazolam and caffeine, followed 24 hours later by chlorzoxazone and debrisoquin (INN, debrisoquine), were administered before (baseline) and at the end of supplementation. Presupplementation and postsupplementation phenotypic trait measurements were determined for CYP3A4, CYP1A2, CYP2E1, and CYP2D6 by use of 1-hydroxymidazolam/midazolam serum ratios (1-hour sample), paraxanthine/caffeine serum ratios (6-hour sample), 6-hydroxychlorzoxazone/chlorzoxazone serum ratios (2-hour sample), and debrisoquin urinary recovery ratios (8-hour collection), respectively. The content of purported "active" phytochemicals was determined for each supplement.**

Results: **Comparisons of presupplementation and postsupplementation phenotypic ratios suggested that these particular supplements had no significant effect on CYP1A2, CYP2D6, CYP2E1, or CYP3A4 activity. Phytochemical profiles indicated that *C aurantium* was devoid of the CYP3A4 inhibitor 6′,7′-dihydroxybergamottin. Quantities of fatty acids, flavonolignans, and cichoric acid were consistent with label claims for saw palmetto, milk thistle, and *E purpurea*, respectively.**

Conclusions: **Botanical supplements containing *C aurantium*, milk thistle, or saw palmetto extracts appear to pose a minimal risk for CYP-mediated herb-drug interactions in humans. Although the effects of *E purpurea***

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on CYP activity were minor, further study into the interaction potential of this botanical is merited. (Clin Pharmacol Ther 2004;76:428-40.)

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Botanical supplements are consumed by a substantial percentage of the US population. Surveys currently indicate that 30% to 49% of all consumers have used some type of dietary supplement in the past 12 months and that 24% regularly use botanical products.[1-4] More recent examinations indicate that as many as 16% of prescription drug users consume herbal dietary supplements.[5] With the widespread use of herbal dietary supplements, the risk of herb-drug interactions is a growing medical concern. This is particularly so because fewer than 40% of patients disclose their herbal supplement usage to health care providers and many physicians are unaware of the potential for herb-drug interactions.[3]

Many botanical dietary supplements contain pharmacologically active phytochemicals that, when consumed concomitantly with conventional medications, may result in pharmacokinetic and/or pharmacodynamic herb-drug interactions.[6-9] Pharmacokinetic herb-drug interactions likely stem from herb-mediated modulation of cytochrome P450 (CYP) enzymes or transporter proteins (eg, P-glycoprotein, organic anion transporter proteins, and organic cation transporter proteins). The botanical supplement most recognized for interacting with prescription medications is St John's wort (*Hypericum perforatum*). Hyperforin, a phytochemical present in St John's wort, is a ligand for the orphan nuclear receptor SXR (steroid xenobiotic receptor) and is responsible for the induction of *CYP3A4* and *MDR1* gene expression. As a result, St John's wort can reduce the effectiveness of many medications including cyclosporine (INN, ciclosporin),[10] indinavir,[11] midazolam,[12] simvastatin,[13] irinotecan,[14] and oral contraceptives.[15] In vitro studies suggest that other botanical supplements may also pose a risk for herb-drug interactions.[16,17]

Recently, a rapid and reliable in vivo screening method that uses single–time point phenotypic metabolic ratios was described for identifying botanical supplements capable of modulating CYP activity.[18] Although not intended to supplant serial concentration-time profiles, phenotypic metabolic ratios can provide reasonable estimates of probe drug clearance, thereby allowing multiple CYP enzymes and multiple botanical supplements to be evaluated in vivo by use of a limited blood-sampling scheme.[18] This method was used to confirm the inductive effect of St John's wort on CYP3A4, and it revealed similar effects for St John's wort on CYP2E1. Among other supplements evaluated with this technique (garlic oil, *Panax ginseng*, and *Ginkgo biloba*), only garlic oil appeared to significantly inhibit CYP2E1. No significant modulation of CYP1A2, 2D6, or 3A4 activity was observed for garlic oil, *P ginseng*, or *G biloba*; these results were later confirmed by other investigators using more traditional assessments of probe drug clearance (ie, area under the curve).[19-21]

In vivo assessments of other top-selling botanical supplements are essential for predicting possible CYP-mediated interactions. In this report we describe, for the first time in humans, the effects of long-term administration of *Citrus aurantium* and milk thistle extracts on CYP1A2, CYP2D6, CYP2E1, and CYP3A4 activity and confirm the effects of *Echinacea purpurea* and saw palmetto on these enzymes using single–time point phenotypic trait measurements. *C aurantium* (also known as Seville orange, bitter orange, or Zhi Shi), a component found in many "Ephedra-free" weight-loss supplements, is a natural source of various furanocoumarins that have been shown to inhibit intestinal CYP3A4 activity.[22,23] *E purpurea* is currently the top-selling dietary supplement in the United States and is touted for its immune-stimulating properties. Milk thistle (*Silybum marianum*) and saw palmetto (*Serenoa repens*) are also among the most popular botanical supplements and are used to promote "healthy liver function" and "good prostate health," respectively. Accordingly, these 4 supplements are likely to be consumed in conjunction with various conventional medications, making knowledge of their herb-drug interaction potential clinically important.

METHODS

Study subjects. The study protocol was approved by the University of Arkansas for Medical Sciences Human Research Advisory Committee (Little Rock, Ark), and all participants provided written informed consent

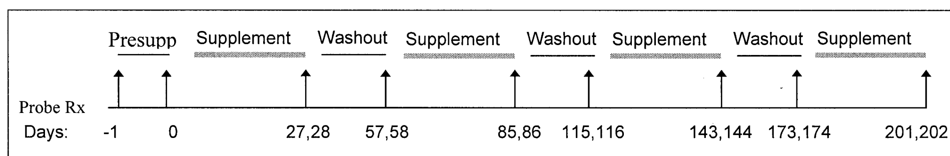

Fig 1. Supplementation and washout scheme. *Arrows* indicate days of probe drug administration.

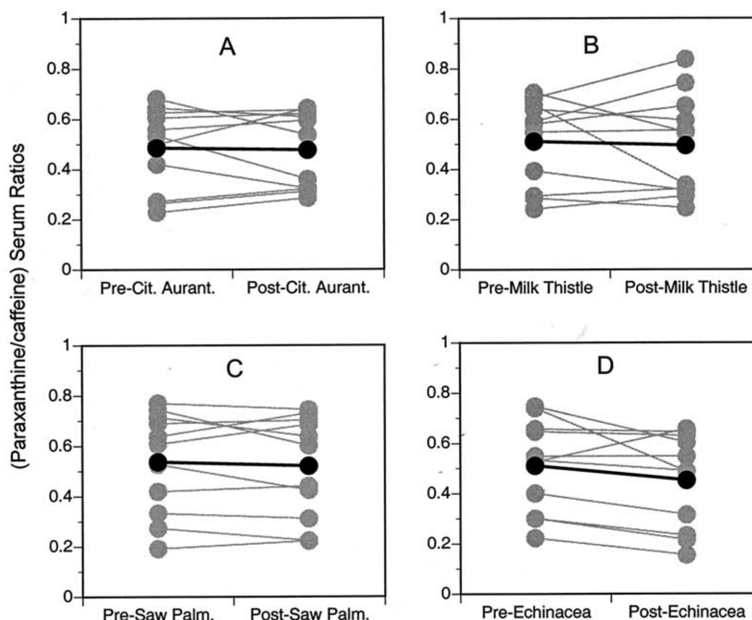

Fig 2. Comparison of presupplementation and postsupplementation phenotypic ratios (paraxanthine/caffeine) for CYP1A2: *Citrus aurantium* (Cit Aurant) (**A**), milk thistle (**B**), saw palmetto (Saw Palm) (**C**), and *Echinacea purpurea* (Echinacea) (**D**). *Gray circles*, Individual values; *black circles*, group means.

before starting the study. Twelve young adults (6 women, 6 men) (mean age [± SD], 25 ± 3.0 years; mean weight, 71.9 ± 14.4 kg) participated in the study, and all subjects were in good health as indicated by medical history, routine physical examination, and clinical laboratory testing. All subjects were extensive metabolizers of CYP2D6 as confirmed by debrisoquin (INN, debrisoquine) urinary recovery screenings.[18] All subjects were nonsmokers, ate a normal diet, and were not users of botanical dietary supplements. Excluding oral contraceptive use (all women), subjects were not taking prescription or nonprescription medications. All female subjects had negative pregnancy test results at baseline. Female subjects continued previously prescribed oral contraceptive therapy. All subjects were instructed to use a barrier method of contraception during the study, in addition to any prescribed oral contraceptive. All subjects were asked to abstain from alcohol, caffeine, fruit juices, cruciferous vegetables, and charbroiled meat throughout the study. Adherence to these restrictions was further emphasized 5 days before each probe drug administration. Subjects were also asked to refrain from taking prescription and nonprescription medications during supplementation periods, and any medication use during this time was documented. Documentation of compliance to these restrictions was achieved through the use of a food/medication diary.

Supplementation and phenotyping procedure. The ability of extracts of *C aurantium*, *E purpurea*, milk thistle, and saw palmetto to modulate human CYP activity was evaluated individually on 4 separate occa-

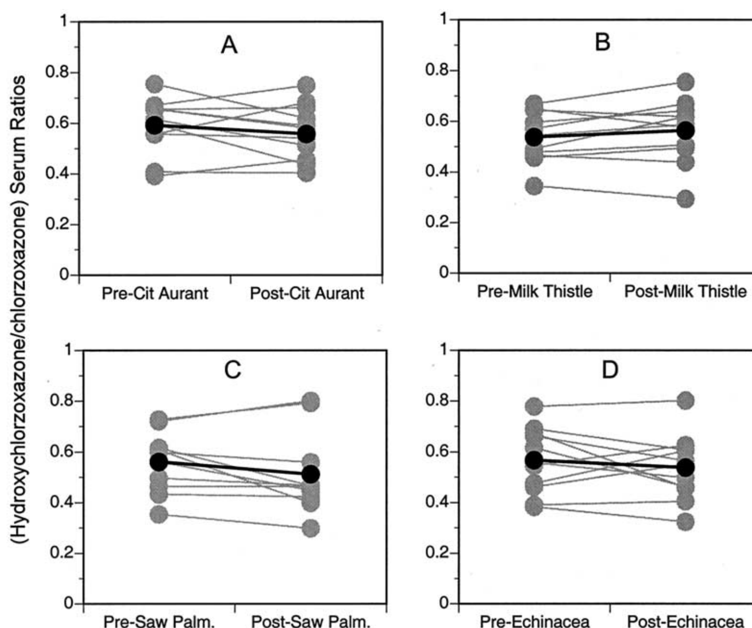

Fig 3. Comparison of presupplementation and postsupplementation phenotypic ratios (6-hydroxychlorzoxazone/chlorzoxazone) for CYP2E1: *C aurantium* (**A**), milk thistle (**B**), saw palmetto (**C**), and *E purpurea* (**D**). *Gray circles*, Individual values; *black circles*, group means.

sions in each subject. This was an open-label study randomized for supplementation sequence. Each supplementation period lasted 28 days and was followed by a 30-day washout period. This randomly assigned sequence of supplementation followed by washout was repeated until each subject had received all 4 botanical supplements (Fig 1). Single lots of *E purpurea*, milk thistle, and saw palmetto botanical supplements were purchased from the same vendor (Wild Oats Markets, Inc, Boulder, Colo). The *C aurantium* supplement (Synephrine, lot No. 88291) was a product of General Nutrition Corp (Pittsburgh, Pa). Product labels were followed regarding the administration of *E purpurea* (800 mg, twice daily, no standardization claim), milk thistle (175 mg, twice daily, standardized to 80% silymarins), saw palmetto (160 mg, twice daily, standardized to 85% to 95% fatty acids and sterols), and *C aurantium* (350 mg, twice daily, standardized to 4% synephrine). Telephone and electronic mail reminders were used to facilitate compliance, and pill counts and supplementation usage records were used to verify compliance.

CYP1A2, CYP2D6, CYP2E1, and CYP3A4 phenotypes were assessed before (days −1 and 0) and at the end of each supplementation phase (days 27 and 28) (Fig 1). Forty-eight hours before supplementation (day −1), each subject received an oral dose of caffeine (100 mg, oral solution; Mallinckrodt Baker, Inc, Paris, Ky) and midazolam (8 mg; Bedford Laboratories, Bedford, Ohio). Blood samples (10 mL) were collected at 1 and 6 hours after probe drug administration and separated by centrifugation (1133*g*) to obtain serum for determining CYP3A4 and CYP1A2 activity. To avoid potential interference from midazolam and caffeine, CYP2E1 and CYP2D6 phenotypes were assessed 24 hours later.[24] The day before supplementation (day 0), each subject emptied his or her bladder before receiving oral doses of chlorzoxazone (250 mg; Watson Laboratories, Corona, Calif) and debrisoquin (5 mg, oral solution; Sigma-Aldrich Co, St Louis, Mo). Blood samples were then obtained at 2 hours and urine was collected for 8 hours, at which time the volume was recorded and a 10-mL aliquot stored for analysis. All samples were stored frozen at −70°C until analyzed. Phenotypes were again assessed on supplementation days 27 (CYP1A2 and CYP3A4) and 28 (CYP2D6 and CYP2E1). The CYP modulatory capability of each botanical supplement was evaluated by comparing individual differences in phenotype before and at the end of 28 days of supplementation.

Analytic methods. Serum concentrations of caffeine and paraxanthine were quantified by HPLC with ultraviolet absorbance detection according to the method of Holland et al.[25] Chlorzoxazone and 6-hydroxychlor-

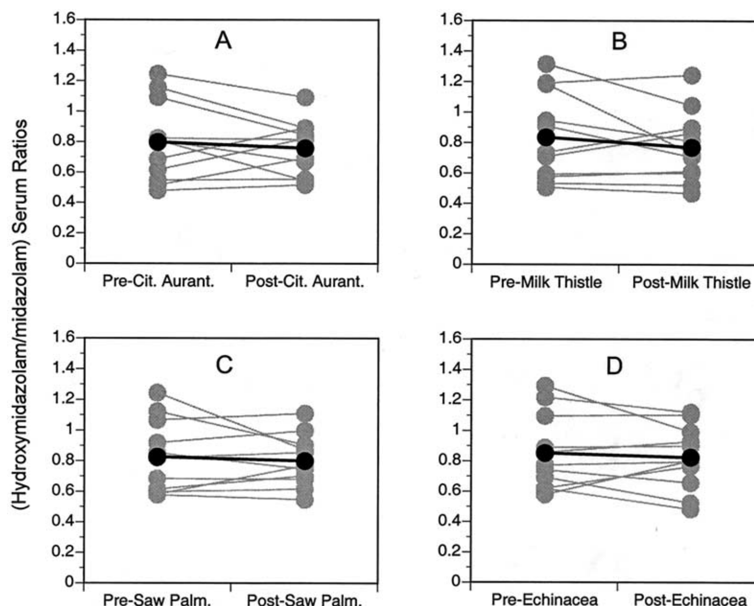

Fig 4. Comparison of presupplementation and postsupplementation phenotypic ratios (1-hydroxymidazolam/midazolam) for CYP3A4: *C aurantium* (**A**), milk thistle (**B**), saw palmetto (**C**), and *E purpurea* (**D**). *Gray circles*, Individual values; *black circles*, group means.

zoxazone serum concentrations were measured by HPLC by use of ultraviolet absorbance detection as previously described by Frye and Stiff.[26] The HPLC method with fluorescence detection described by Frye and Branch[27] was used for the quantitation of debrisoquin and 4-hydroxydebrisoquin in urine. A previously described modification of the HPLC method of Sautou et al[28] was used to determine serum concentrations of midazolam and 1-hydroxymidazolam.[18] To optimize the recovery of 6-hydroxychlorzoxazone and 1-hydroxymidazolam, serum samples (250 μL) for these probe drugs were incubated with β-glucuronidase (250 μL, 1800 U/mL) for 2.5 hours at 37°C.

Phenotype assessment. The justification of specified time points for obtaining metabolite/parent serum ratios to estimate probe drug clearance has been addressed previously.[18] Serum ratios of 1-hydroxymidazolam/midazolam determined 1 hour after dosing were used to estimate CYP3A4 activity. CYP1A2 phenotypes were determined from paraxanthine/caffeine serum ratios obtained at 6 hours. CYP2E1 activity was estimated from 6-hydroxychlorzoxazone/chlorzoxazone serum ratios obtained 2 hours after dosing, and CYP2D6 activity was assessed by use of 8-hour debrisoquin urinary recovery ratios (4-hydroxydebrisoquin/[debrisoquin + 4-hydroxydebrisoquin]).

Phytochemical analysis. The phytochemical content of each supplement was independently analyzed for specific "marker compounds" by HPLC. Flavonolignan content of milk thistle was quantitated by use of the method of Wallace et al.[29] Fatty acid of saw palmetto was quantitated by use of the method of Ganzera et al.[30]

E purpurea was analyzed for cichoric acid, echinacoside, and chlorogenic acid at the National Center for Natural Products Research (University of Mississippi, University, Miss) by use of a proprietary gradient HPLC method similar to that described by Molgaard et al.[31] In brief, chromatographic separations were performed on a Lichrosphere RP-18 column (150 × 4.6 mm; 5-μm particle size) (Phenomenex, Torrance, Calif) by use of a Waters 2695 Alliance Separations Module with a 996 photodiode array detector operated at a wavelength of 330 nm (Waters Corp, Milford, Mass). A mobile phase consisting of 0.1% phosphoric acid in water (A) and 0.1% phosphoric acid in acetonitrile (B) was pumped at a flow rate of 1 mL/min. The gradient elution was as follows: 95% A/5% B to 60% A/40% B in 20 minutes and then to 100% B in 5 minutes. Each run was followed by a 5-minute wash with 100% B and an equilibration period of 10 minutes. *E purpurea* capsules were weighed and transferred to 15-mL polystyrene conical tubes. Three milliliters of methanol was added, and the samples were sonicated for 15 minutes. After centrifugation of the sonicated sample, the supernatant was pipetted into a 10-mL volumetric flask. This procedure was repeated twice,

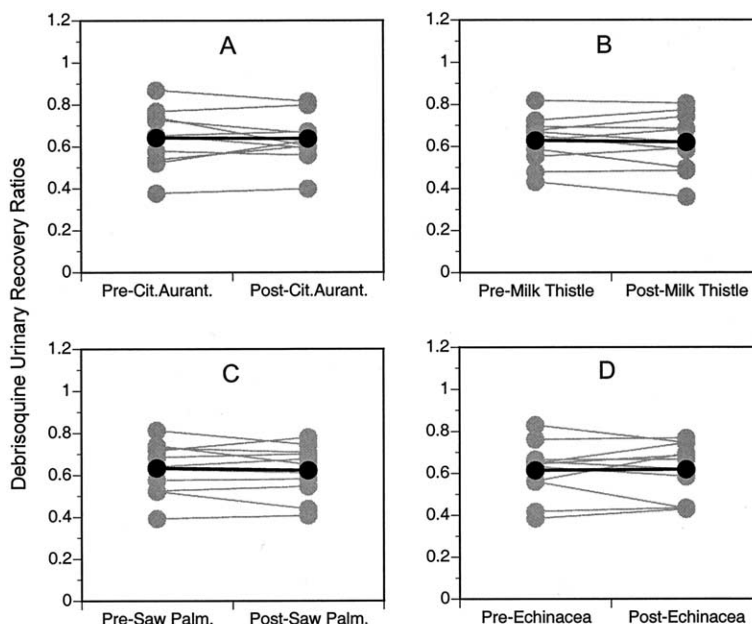

Fig 5. Comparison of presupplementation and postsupplementation phenotypic ratios (8-hour debrisoquin urinary recovery ratios) for CYP2D6: *C aurantium* (**A**), milk thistle (**B**), saw palmetto (**C**), and *E purpurea* (**D**). *Gray circles*, Individual values; *black circles*, group means.

and the samples were diluted to volume with methanol. Each sample was filtered through a 0.45-μm nylon membrane filter, and a 10-μL aliquot was injected onto the HPLC column. Retention times for chlorogenic acid, echinacoside, and cichoric acid were 8.2, 10.1, and 14 minutes, respectively.

C aurantium was analyzed for p-synephrine and octopamine content by use of a modification of the method of Gurley et al.[32] The extraction procedure for capsules containing *C aurantium* extract was identical to that for *Ephedra*-containing supplements described previously.[32] HPLC conditions were identical to those described for quantitation of ephedrine alkaloids, except that the mobile phase consisted of water, acetonitrile, tetrahydrofuran, and phosphoric acid (59.9:38:2:0.1 [vol/vol/vol/vol]). Sodium dodecyl sulfate, an ion-pairing agent, was added to the mobile phase to achieve a final concentration of 5 mmol/L. Retention times for octopamine and p-synephrine were 10.8 and 11.9 minutes, respectively. The assay was linear over a concentration range of 3.1 to 400 μg/mL for both *p*-synephrine and octopamine. Percent recovery of each compound exceeded 95%. Quantitation of the 6′,7′-dihydroxybergamottin content of *C aurantium* extract was performed according to the method of Edwards et al.[33]

Disintegration tests. An absence of botanical-mediated changes in CYP phenotype could stem from products exhibiting poor disintegration or dissolution characteristics. To address this concern, each product was subjected to disintegration testing as outlined in the *United States Pharmacopeia*, 27th revision.[34] The disintegration apparatus consisted of a basket-rack assembly operated at 29 to 32 cycles/min with 0.1N hydrochloric acid (37°C) as the immersion solution. One dosage unit of each supplement was placed into each of the 6 basket assembly tubes. The time required for the complete disintegration of 6 dosage forms was determined. This process was repeated with an additional 6 dosage units to ensure accuracy. Because there are no specifications for the disintegration time of the botanical supplements used in this study, the mean of 6 individual dosage forms was taken as the disintegration time for that particular product. A product (eg, hard gelatin capsule, soft gelatin capsule, or uncoated tablet) was considered completely disintegrated if the entire residue passed through the mesh screen of the test apparatus, except for capsule shell fragments, or if the remaining soft mass exhibited no palpably firm core.

Statistics. A repeated-measures ANOVA model was fit for each phenotype response by use of SAS Proc Mixed software (SAS Institute, Inc, Cary, NC). Be-

Table I. Presupplementation and postsupplementation-phenotypic-ratios (means, 95% confidence intervals)

Phenotypic ratio (CYP)	*Supplement*	*Presupplementation (mean and 95% CI)*	*Postsupplementation (mean and 95% CI)*	*Difference (mean and 95% CI)*
OH-MDZ/MDZ (CYP3A4)	*E purpurea*	0.848 (0.692 to 1.004)	0.816 (0.660 to 0.971)	−0.032 (−0.136 to 0.071)
	Milk thistle	0.836 (0.710 to 1.077)	0.770 (0.661 to 1.028)	−0.066 (−0.251 to 0.153)
	C aurantium	0.799 (0.623 to 0.903)	0.758 (0.592 to 0.853)	−0.041 (−0.153 to 0.092)
	Saw palmetto	0.826 (0.654 to 0.958)	0.800 (0.603 to 0.906)	−0.026 (−0.150 to 0.048)
DMX/CFE (CYP1A2)	*E purpurea*	0.514 (0.426 to 0.593)	0.447 (0.368 to 0.526)	−0.067 (−0.136 to 0.031)
	Milk thistle	0.519 (0.418 to 0.620)	0.494 (0.392 to 0.595)	−0.025 (−0.132 to 0.079)
	C. aurantium	0.467 (0.410 to 0.523)	0.458 (0.401 to 0.514)	−0.009 (−0.077 to 0.059)
	Saw palmetto	0.513 (0.412 to 0.613)	0.509 (0.408 to 0.609)	−0.004 (−0.050 to 0.043)
OH-CZX/CZX (CYP2E1)	*E. purpurea*	0.567 (0.355 to 0.724)	0.538 (0.411 to 0.650)	−0.029 (−0.198 to 0.110)
	Milk thistle	0.569 (0.404 to 0.734)	0.575 (0.409 to 0.740)	0.006 (−0.041 to 0.030)
	C aurantium	0.593 (0.396 to 0.768)	0.558 (0.481 to 0.853)	−0.035 (−0.157 to 0.046)
	Saw palmetto	0.550 (0.381 to 0.719)	0.499 (0.330 to 0.668)	−0.051 (−0.170 to 0.068)
HDEB/(DEB + HDEB) (CYP2D6)	*E purpurea*	0.615 (0.523 to 0.708)	0.623 (0.530 to 0.715)	0.008 (−0.041 to 0.056)
	Milk thistle	0.630 (0.543 to 0.716)	0.624 (0.537 to 0.710)	−0.006 (−0.042 to 0.030)
	C aurantium	0.635 (0.528 to 0.743)	0.632 (0.525 to 0.739)	−0.003 (−0.072 to 0.066)
	Saw palmetto	0.635 (0.548 to 0.721)	0.624 (0.538 to 0.711)	−0.011 (−0.047 to 0.025)

CI, Confidence interval; OH-MDZ/MDZ, 1-hydroxymidazolam/midazolam; DMX/CFE, paraxanthine/caffeine; OH-CZX/CZX, 6-hydroxychlorzoxazone/chlorzoxazone; HDEB/(DEB + HDEB), 4-hydroxydebrisoquin/(debrisoquin plus 4-hydroxydebrisoquin).

cause presupplementation and postsupplementation phenotypic ratios were determined in each subject for all 4 supplements, a covariance structure existed for measurements within subjects. Sex, supplement, and supplement-by-sex terms were estimated for each phenotype by use of a Huynh-Feldt covariance structure fit. If supplement-by-sex interaction terms for a specific phenotypic measure were significant at the 5% level, the focus of the postsupplementation minus presupplementation response was assessed according to sex. If the supplement-by-sex interaction was not statistically significant, responses for both sexes were combined.

In addition, a power analysis was performed to estimate the ability to detect significant post-supplementation minus pre-supplementation effects. All 4 phenotype models obtained at least 83% power at the 5% level of significance to detect a medium Cohen effect size of 0.53 to 0.57 SD units.[35]

RESULTS

General experimental observations. No serious adverse events occurred during the course of the study. Breakthrough bleeding was noted in one female subject during supplementation with milk thistle, which prompted her withdrawal from the study. Another had a mild urinary tract infection develop while taking milk thistle. Minor complaints reported for saw palmetto included night sweats and diarrhea. One subject had a mild rash develop while taking *Echinacea*. Except for the allowed use of oral contraceptives, no significant violations of the dietary and medication restrictions were noted from the food and medication diaries.

Effect of supplementation on CYP phenotype. The effects of long-term *C aurantium*, milk thistle, *E purpurea*, and saw palmetto extract supplementation on CYP phenotypic ratios are shown in Figs 2 through 5 and Table I. Although moderate differences in presupplementation and postsupplementation phenotypic ratios were noted for specific individuals in each group, no statistically significant differences in mean CYP1A2, CYP2E1, CYP2D6, or CYP3A4 phenotypic ratios were observed over the 28-day supplementation period, nor were any significant differences observed in baseline phenotypes. Accordingly, no sex-related differences were noted either. The only perceptible change in mean phenotypic ratios occurred with 6-hour paraxanthine/caffeine values, where an approximate 13% decrease (from 0.514 ± 0.181 to 0.447 ± 0.190) hinted at a possible inhibitory effect of *E purpurea* on CYP1A2 (Fig 2, *D*, and Table I). This minor difference was not statistically significant ($P = .07$) nor was it considered clinically relevant.

Phytochemical content and disintegration testing. HPLC determinations of various “marker” phytochemicals and the daily amount ingested by each subject are shown in Table II. Table III depicts the mean disintegration time for each supplement dosage form, all of which were less than 15 minutes.

Table II. Content of phytochemical marker compounds for botanical supplements.

Supplement	*Compound*	*Content (mg/capsule)*	*Daily dose (mg)*
E purpurea	Cichoric acid	13.7	43.8
	Chlorogenic acid	ND	—
	Echinacoside	ND	—
	Total	13.7	43.8
Saw palmetto	Nonesterified fatty acid		
	Oleic acid	48.3	96.6
	Lauric acid	40.2	80.4
	Myristic acid	15.1	30.2
	Palmitic acid	12.9	25.8
	Linoleic acid	8.2	16.4
	Capric acid	4.1	8.2
	Caprylic acid	3.7	7.4
	Caproic acid	3.1	6.2
	Linolenic acid	2.9	5.8
	Stearic acid	ND	—
	Total	138.5	277
Milk thistle	Silybinin A	15.3	61.2
	Silybinin B	32.3	129.2
	Silychristin	16.2	64.8
	Silydanin	1.6	6.4
	Taxifolin	13.9	55.6
	Total	79.3	317.2
C aurantium	Sympathomimetics		
	p-Synephrine	15.3	30.6
	Octopamine	ND	—
	Total	15.3	30.6
	Furanocoumarins		
	6,7-dihydroxybergamottin	ND	—

ND, Not detected.

DISCUSSION

These findings, coupled with those presented previously for St John's wort, *G biloba*, *P ginseng*, and garlic oil,[18] suggest that single–time point phenotypic ratios provide a practical method for predicting CYP-mediated herb-drug interactions. Even with a limitation of estimated probe drug clearances, its distinct advantage lies in the ability to evaluate multiple CYP enzymes and multiple botanical supplements in vivo while using a limited blood-sampling scheme. The utility of phenotypic ratios for estimating drug clearance and predicting herb-drug interactions has been discussed previously.[18]

Comparisons of presupplementation and postsupplementation single–time point phenotypic ratios suggest that prolonged use of *E purpurea*, saw palmetto, milk thistle, or *C aurantium* extracts poses a minimal risk of producing CYP1A2-, CYP2D6-, CYP2E1-, or CYP3A4-mediated herb-drug interactions in humans. With regard to the effects of *E purpurea* extract on CYP3A4 and CYP2D6, our findings are in general agreement with those of Gorski et al.[36] This conformity occurred despite differences in the type of *E purpurea* products used (root extract in their study versus whole plant extract in our study), duration of supplementation (8 days versus 28 days), and CYP phenotype assessment methodologies (area under the concentration-time curve versus single–

Table III. Disintegration times for supplement dosage forms

Supplement	*Dosage form*	*Disintegration time (min)*
E purpurea	Hard gelatin capsule	10.4
Saw palmetto	Soft gelatin capsule	13.5
Milk thistle	Hard gelatin capsule	5.7
C aurantium	Hard gelatin capsule	10.4

time point phenotypic ratio). Like the results of Gorski et al, our results indicated that midazolam oral clearance was not significantly altered by *E purpurea* supplementation. Gorski et al observed, however, that *E purpurea* augmented midazolam systemic clearance (approximate 34% increase) through an induction of hepatic CYP3A4 activity while inhibiting intestinal CYP3A4 activity. These seemingly contradictory effects in the two organs may have been at work in the current study, but confirmation of such distinctions is beyond the capability of the single–time point approach. This ostensibly complex effect of *E purpurea* on intestinal and hepatic CYP3A4 may stem from a variety of reasons, one being the multiplicity of biologically active phytochemicals present in *Echinacea* species.[37,38]

Gorski et al[36] also noted a significant reduction in the oral clearance (dose/area under the concentration-time curve extrapolated to infinity) of the CYP1A2 probe caffeine, as well as in 6-hour paraxanthine/caffeine serum ratios, implying an inhibitory effect of *E purpurea* on this enzyme. A comparison of 6-hour paraxanthine/caffeine serum ratios between the 2 studies reveals very similar results (0.59 to 0.53 in their study versus 0.51 to 0.45 in our study); however, a greater degree of interindividual variability was noted in their study. Greater variability among subjects may not only explain the difference in study results but could suggest that certain individuals are more susceptible to *Echinacea*-mediated effects on CYP1A2.

Disparity among phytochemical profiles for the 2 *E purpurea* products may also have contributed to the subtle differences in the studies. Whereas the *E purpurea* root extract used by Gorski et al[36] purportedly contained greater than 1% phenols, quantities of individual hydrophilic phenolic compounds (eg, cichoric acid, chlorogenic acid, and echinacoside) were not independently confirmed. The *E purpurea* product used in our study was a whole plant extract containing only cichoric acid and no chlorogenic acid or echinacoside (Table I). Such findings are not unexpected, as phytochemical profiles can vary considerably among commercial *Echinacea* products.[31,39] Cichoric acid is the principal phenolic compound found in *E purpurea*, whereas only minor quantities of chlorogenic acid are found in this species.[37,40] Echinacoside, on the other hand, is the main phenolic of *E angustifolia* and *E pallida*.[40] Other phytochemicals unique to *E purpurea* include caftaric acid,[40] a number of lipophilic alkylamides,[31,37] numerous volatile components,[41] and polysaccharides.[38] Although in vitro examinations of both *E purpurea* extracts and cichoric acid have demonstrated mild inhibition of CYP3A4,[42] cichoric acid is an unlikely candidate for the mixed effects observed by Gorski et al, as this compound does not readily cross the intestinal epithelial barrier.[43] Certain unique alkylamides present in *E purpurea*, however, may be accountable, as they readily cross the intestinal barrier and reach the systemic circulation in measurable concentrations.[43,44]

The results of the 2 studies suggest that *E purpurea* has a relatively low potential for producing CYP-mediated herb-drug interactions; however, the disparity in intestinal and hepatic CYP3A4 activity observed by Gorski et al[36] implies that CYP3A4 substrates with comparatively high bioavailability may be more susceptible to *Echinacea*-mediated interactions. Therefore more in vivo studies are warranted before the interaction potential of *E purpurea* can be fully characterized. Until such time, its concomitant use with conventional medications should be discouraged.

Saw palmetto may be an effective alternative treatment for mild benign prostatic hyperplasia, with an ability to improve urologic symptoms and urine flow measures comparable to that of finasteride.[45] Consequently, men treated with prescription medications for benign prostatic hyperplasia often self-medicate with saw palmetto.[4] Conceivably, such practices could increase the likelihood of drug interactions with saw palmetto; however, this does not appear to be the case. Using a conventional pharmacokinetic study design for alprazolam and metabolic urinary ratios for dextromethorphan, Markowitz et al[46] showed that saw palmetto did not significantly modulate CYP3A4 or CYP2D6 activity. The 1-hour hydroxymidazolam/midazolam serum ratios and 8-hour debrisoquin urinary recovery ratios presented here confirm that CYP3A4 and CYP2D6 activities are unaffected by saw palmetto supplementation. This agreement arose despite differences in the daily dose of fatty acids (197 mg versus 277 mg) administered, duration of supplementation (14 days versus 28 days), and CYP phenotype assessment methodologies (area under the concentration-time curve versus single–time point phenotypic ratio). In addition, our study also demonstrates that saw palmetto has no significant modulatory effects on CYP1A2 and CYP2E1. Therefore the concordant results of our study and that of Markowitz et al establish saw palmetto as an unlikely candidate for CYP-mediated herb-drug interactions.

Milk thistle's purported hepatoprotectant properties are believed to be a result of silymarin, a mixture of flavonolignans (silibinin A, silibinin B, silichristin, silidianin, taxifolin) extracted from the seeds of *Silybum marianum*. Silymarin has a good safety profile, but its

mechanism of action and drug interaction potential are unclear.[47] In vitro investigations implicate milk thistle extract and/or silibinin as inhibitors of human CYP3A4, CYP2C9, CYP2D6, and CYP2E1.[48-51] Of the enzymes investigated, only CYP3A4 and CYP2C9 were inhibited at concentrations similar to those observed in vivo. Reports detailing the pharmacokinetics of silibinin in humans have yielded varied results.[47,52] Oral administration of silymarin extract in doses from 120 to 360 mg produced serum concentrations of silibinin and its glucuronide and sulfate conjugates in the range of 100 to 1400 ng/mL, whereas bile concentrations reached 100 times those of serum, indicating extensive biliary secretion.[47] Thus, when administered orally, concentrations of silymarin components may be sufficient to compete for CYP binding sites in the liver and gut wall.

In vivo evidence for CYP-mediated milk thistle interactions, however, is less compelling. Administration of silymarin (Legalon [Madaus AG, Köln, Germany], 70 mg 3 times daily) for 28 days to healthy volunteers had no effect on the pharmacokinetics of aminopyrine or phenylbutazone, two nonspecific CYP probes.[53] After 21 days of milk thistle extract administration (153 mg silymarin, 3 times daily), no clinically significant changes in the pharmacokinetics of indinavir (a CYP3A4 substrate) were noted in humans.[54] A second study of healthy volunteers also found that 20 days of silymarin ingestion (160 mg 3 times daily) had no effect on the pharmacokinetics of indinavir.[55] Results of the current study verify that milk thistle does not appear to modulate human CYP1A2, CYP2D6, CYP2E1, and CYP3A4 in vivo and thus poses no significant interaction potential for substrates of these enzymes. This apparent lack of in vitro–in vivo correlation may stem from poor bioavailability, large interindividual variations in silibinin absorption, lower CYP binding affinities of silibinin conjugates, interproduct variability in silymarin content, or poor dissolution characteristics of milk thistle dosage forms.[47,50,56] The daily dose of silymarin administered in this study (317 mg) (Table II) and the dosage form's rapid disintegration (<6 minutes) (Table 3), however, argue against these last 2 options.

A recent Food and Drug Administration decision removing Ephedra-containing dietary supplements from the marketplace because of unreasonable risks for serious adverse health events has led to an influx of "Ephedra-free" products containing *C aurantium*.[57] Accordingly, supplement manufacturers have embraced *C aurantium*, a natural source of the sympathomimetic agents p-synephrine and octopamine, as a substitute for Ephedra and the ephedrine alkaloids present in that controversial supplement. This substitution has raised concerns about the drug interaction potential of *C aurantium*–containing supplements. These concerns arise from the potent inhibitory effects of Seville orange juice on intestinal CYP3A4.[22,23,33,58] Seville orange juice contains various furanocoumarins that have been implicated as mechanism-based inhibitors of CYP3A4.[59,60] One of these compounds, 6′,7′-dihydroxybergamottin, is present in large quantities in Seville orange juice.[58] Figs 2 through 5, however, indicate that daily administration of 700 mg of *C aurantium* extract for 28 days failed to register any significant changes in CYP phenotype. An absence of 6′,7′-dihydroxybergamottin from the extract, a finding confirmed by HPLC analysis (Table II), is the most likely explanation for these results. Most *C aurantium* extracts are prepared by extracting the fruit with hot water,[61] and given the extremely poor water solubility of most furanocoumarins, it is not surprising that the product studied was devoid of 6′,7′-dihydroxybergamottin (*p*-synephrine, a water-soluble amine, was present in the extract). Therefore, unlike their whole juice counterparts, *C aurantium* extracts appear to be, at best, weak inhibitors of intestinal CYP3A4 activity.

In conclusion, the present findings provide additional support for the use of single–time point phenotypic ratios as a practical means of predicting CYP-mediated herb-drug interactions. They also confirm that saw palmetto poses little risk of interfering with conventional medications whose metabolism is mediated by CYP1A2, CYP2D6, CYP2E1, or CYP3A4. Similar conclusions can also be made for extracts of milk thistle and *C aurantium*, and these studies represent the first of their kind for these 2 supplements. The highly popular supplement *E purpurea* has little modulatory effect on CYP2D6 and CYP2E1, but its influence on CYP1A2 and CYP3A4, though seemingly minor, merits further study. Therefore concomitant administration of *E purpurea* with conventional medications is not recommended.

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