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Effects of Herbal Products and their Constituents on Human Cytochrome P4502E1 Activity

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Abstract

Ethanol extracts from fresh *Echinacea purpurea* and *Spilanthes acmella* and dried *Hydrastis canadensis* were examined with regard to their ability to inhibit cytochrome P4502E1 mediated oxidation of p-nitrophenol in vitro. In addition, individual constituents of these extracts, including alkylamides from *E. purpurea* and *S. acmella*, caffeic acid derivatives from *E. purpurea*, and several of the major alkaloids from *H. canadensis*, were tested for inhibition using the same assay. *H. canadensis* (goldenseal) was a strong inhibitor of the P450_{2E1}, and the inhibition appeared to be related to the presence of the alkaloids berberine, hydrastine and canadine in the extract. These compounds inhibited 2E1 with K_i values ranging from 2.8 μM for hydrastine to 18 μM for berberine. The alkylamides present in *E. purpurea* and *S. acmella* also showed significant inhibition at concentrations as low as 25 μM, whereas the caffeic acid derivatives had no effect. Commercial green tea preparations, along with four of the individual tea catechins, were also examined and were found to have no effect on the activity of P450_{2E1}.

Keywords

Echinacea; cytochrome P4502E1; Hydrastis; Goldenseal; Spilanthes; Green Tea

Introduction

Cytochrome P450_{2E1} is an alcohol inducible P450 isoform that has been implicated in the generation of reactive oxygen species such as superoxide and hydrogen peroxide and may mediate the toxic effects of a variety of xenobiotic compounds (Gorsky et al., 1984; Guengerich et al., 1991; Castillo et al., 1992). This isoform has been identified in a wide range of tissue types outside the liver, including, intestines (Subramanian and Ahmed, 1995), tongue (Yang et al., 2003), kidney, and nasal mucosa (Ding et al., 1986). The widespread distribution of this isoform and its potential role in activating toxins, along with the observed induction by exposure to alcohol, suggest that compounds with the ability to inhibit P450_{2E1} may be useful in the prevention of deleterious effects of various chemical toxins that are activated by this enzyme. For example, Xu et. al. (2003) have shown that exposure of human hepatoma cells

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(HepG2) to arachidonic acid results in the generation of hydrogen peroxide, and that cells overexpressing cytochrome P450_{2E1} produce elevated levels of hydrogen peroxide, implicating this P450 isoform in its formation. The hydrogen peroxide produced in these cells is genotoxic due to resulting lipid peroxidation. Moreover, treatment of the cells with the retinoid lycopene protected the cells from the P450_{2E1}-derived peroxide. Others have also demonstrated that inhibition of cytochrome P450_{2E1} can lead to reduction in the genotoxicity of other xenobiotics whose toxicity is mediated by this enzyme (Hammond and Fry, 1997).

Herbal extracts hold promise for use in strategies for inhibiting the metabolic activation of toxic xenobiotics. Such extracts contain a diverse array of chemical species, each with the potential to act on specific cytochrome P450 enzymes in an inhibitory manner. Many examples of herbs or food products that interact with cytochrome P450 enzymes exist in the literature (Brady et al., 1988; Guengerich and Kim, 1990; Subehan et al., 2006; Koul et al., 2000), and in some instances, drug interactions have been observed (Bailey et al., 1998; Wentworth et al., 2000). However, the complex nature of the extracts is a problem from a regulatory standpoint and in understanding the biochemical affects they exert on xenobiotic metabolizing systems. Thus, it is important not only to recognize the inhibitory effect of a complete extract, but to also identify the individual components of the extract that contribute to the observed inhibitory effect.

The herbal products *Echinacea purpurea*, *Hydrastis canadensis*, green tea, and *Spilanthes acmella* are popular products that are widely used for medicinal purposes. The effects of some of these products on drug metabolism in various tissue types have been studied (Budzinski et al., 2000; Yale and Glurich, 2005; Yang and Raner, 2005; Gorski et al., 2004; Chatterjee and Franklin, 2003). However, the ethanol inducible cytochrome P450_{2E1} enzyme is often neglected in such pharmacologically driven studies. Consequently, little is known regarding the potential interaction of cytochrome P450_{2E1} with these natural products. Given the possible role of P450_{2E1} in the generation of reactive oxygen species in the liver, and the resulting oxidative stress associated with these species, interactions that could reduce P450_{2E1} activity under certain conditions may prove beneficial.

In the current study, we have examined preparations of four different medicinal plants, cone flower (*Echinacea purpurea*), toothache plant (*Spilanthes acmella*), goldenseal (*Hydrastis canadensis*) and green tea (*Camellia sinensis*). Their abilities to inhibit cytochrome P450_{2E1} from human liver microsomes and from an in vitro expression system have been evaluated. In addition, we have selected several major classes of chemical species found in each of the extracts and tested the individual compounds with respect to their inhibitory properties on the human P450_{2E1} isoform.

Materials and Methods

Chemicals and enzymes

All of the chemicals used in this study except for the alkylamides from *Echinacea purpurea* and *Spilanthes acmella* were purchased from commercial suppliers Sigma Aldrich or Acros Chemical Companies. *E. purpurea* fresh roots were purchased from Pacific Botanicals (Williams, OR) and *S. acmella* fresh plants were purchased from Horizon Herbs (Williams, OR). Ethanolic extracts (95 % and 33%) were prepared from this plant material according to published procedures (Cech et al., 2006a). Dried roots of *Hydrastis canadensis* were also obtained from Horizon Herbs and were extracted at a ratio of 1:5 (1 mL solvent: 5 g powdered roots) in 50% ethanol: 50% water. A commercial green tea extract (standardized to contain 50% polyphenols) manufactured by Spring Valley (Bohemia, NY) was used.

Purification of alkylamides from *S. acmella* and *purpurea*

Alkylamides were separated from the 95% ethanol extracts of *S. acmella* and *E. purpurea*. Concentrated extract (100 mL) was diluted to 200 mL with de-ionized water. A 35 g C18 reverse phase extraction cartridge was equilibrated with 100 mL of a 50% ethanol:50% H₂O solution. The diluted *E. purpurea* solution was loaded onto the cartridge at a flow rate of 7 mL/min. After the entire 200 mL had passed through, the cartridge was washed with 100 mL of 50% ethanol:50% H₂O. The eluent was collected in 25 mL fractions and stored for later analysis. Three subsequent elutions were carried out in the same manner. The mobile phase for these three elutions consisted of 55% ethanol: 45% H₂O, 60% ethanol: 40% H₂O and 70% ethanol:30% H₂O, respectively. A total of four-25mL fractions were collected from each of the washing steps for a total of 16 fractions. At this point, each of the fractions was analyzed by HPLC to identify those containing alkylamides. Fractions containing significant amounts of the alkylamides were then submitted to HPLC purification using a semi-preparative C18 column (250 × 10 mm) with an injection volume of up to 5 mL. The column was first equilibrated in 50% acetonitrile containing 0.1% trifluoroacetic acid and the sample was analyzed using the same mobile phase with a flow rate of 1.5 mL/min and a detection wavelength of 200 nm. Individual peaks from the chromatogram were collected and the mobile phase was evaporated. Identity of the alkylamides in the fractions was verified using an LC/MS assay described previously (Cech et al., 2006a). An identical procedure was used to purify several *S. acmella* alkylamides.

Quantification of alkylamides in *E. purpurea* and *S. acmella* extracts

Since standards for each of the alkylamides are not commercially available, a single compound (dodeca-2E,4E,8Z,10Z-tetraonic acid isobutylamide) was purchased from Chromadex (Santa Anna, CA) and used as a standard for quantification of all of the isobutylamides isolated. It was assumed that the spectrophotometric properties of all of the alkylamides would be similar enough for this approach to provide a reasonable estimate of the concentrations of each in the complex extracts.

Cytochrome P450_{2E1} assay

Cytochrome P450_{2E1} activity was monitored using the substrate p-nitrophenol in human liver microsomes and expressed cytochrome P450_{2E1}. The P450_{2E1} assay used in this laboratory has been reported previously, and was used without modification for control reactions (Larson et al., 1991). The inhibitory properties of the extracts and individual components were determined by including a known quantity of the inhibitor in the P450_{2E1} assay and monitoring the effects on V_{max} and K_m for the reaction. In all cases, if ethanol or methanol was present in the stock solution of inhibitor, the solvent was evaporated, and the residue was redissolved in the assay buffer prior to adding the enzyme and remaining components, since ethanol is a known inhibitor of P450_{2E1}. This also ensured that the inhibitor was soluble in the reaction mixture at the concentrations used.

Quantification of individual components in various extracts

The four major catechins in green tea extract, EGCG, EC, EGC and ECG were quantified in a sample of the extract using a three point calibration curve as described previously (Lee et al., 2000). Caffeic acid derivatives, caftaric acid, chlorogenic acid and cichoric acid were analyzed in the 33% ethanolic *E. purpurea* extract using HPLC separation followed by ESI-MS for detection and quantification (Sasagawa et al., 2006). The HPLC system used was an HP1100 by Agilent with a C18 (50 × 2.1, 3.0 μm particle size) column. Injection volume was 10 μL and flow rate set at 0.2 mL/min. Calibration curves were generated using a mixture of standard compounds purchased from Chromadex, Inc (Santa Anna, CA, USA). The alkaloids berberine, hydrastine and canadine were quantified in the goldenseal extract using HPLC and ESI-MS

with a similar method to that previously published for *Echinacea* (Sasagawa et al., 2006). Standards of the alkaloids were purchased from Sigma Aldrich in Saint Louis, MO (hydrastine and berberine) and from Sequoia Research in the UK (canadine). The method of analysis for goldenseal differed from that for *Echinacea* only by the use of a different gradient for separation as follows (where A = 20 mM ammonium acetate in 90:10 water: acetonitrile adjusted to pH 4.5 with acetic acid, and B = acetonitrile): t = 0 to 5 min, 15% B; t = 5 to 20 min, 15% B to 25% B; t = 20–23 min, 25% B, t = 23–25 min, 15% B.

RESULTS AND DISCUSSION

Interaction of *E. purpurea* and *S. acmella* Extracts with P450_{2E1} in vitro

Table 1 lists the inhibitory properties of *E. purpurea* and *S. acmella* extracts on human cytochrome P450_{2E1} in both liver microsomes and baculovirus expressed enzyme. Extracts of *E. purpurea* root in 95% ethanol significantly inhibited the activity of cytochrome P450_{2E1} in human liver microsomes when present at 0.4% full strength (2.0 µL of extract in 500 µL reaction). Inhibition was approximately 30% using a *p*-nitrophenol concentration of 0.020 mM. Likewise, *p*-nitrophenol oxidation in baculovirus expressed human P450_{2E1} and P450 reductase was inhibited 30% under the same conditions. The 95% ethanol extract from whole flowering *S. acmella* plants was much less potent in its inhibition of P450_{2E1}. For example, 8% extract (40 µL in a 500 µL reaction) was required in human liver microsomes to achieve 40% inhibition under identical conditions. Interestingly, greater than 50% inhibition was observed in the expressed P450_{2E1} using the same conditions. Based on the more potent inhibition seen with the *E. purpurea* extract, it may be concluded that either components in the *E. purpurea* extract had a higher binding affinity to the P450_{2E1} enzyme, or the *Echinacea* extract had a higher content of components with the ability to interact with P450_{2E1}. Additional experiments were carried out using a 33% ethanolic extract of the *E. purpurea* roots. Inhibition of P450_{2E1} by the 33% extract was much less potent, where no inhibition was seen when 2 µL of the extract was used in a 500 µL reaction using human liver microsomes or expressed P450.

E. purpurea extracts prepared using solvents with high ethanol content generally contain significant quantities of hydrophobic alkylamides, while *E. purpurea* extracts prepared in ethanol/water mixtures contain greater quantities of the more hydrophilic caffeic acid derivatives (Sasagawa et al., 2006). The higher inhibitor activity of the extract with higher ethanol content suggests that alkylamides rather than caffeic acid derivatives are likely inhibitory compounds in *E. purpurea*. This hypothesis is supported by the observation that neither of the two main caffeic acid derivatives, caftaric acid and cichoric acid, showed any inhibition of P450_{2E1} at concentrations as high as 0.400 mM (data not shown). In vivo studies by Gorski et al. have shown that *E. purpurea* extracts reduced the oral clearance of CYP1A2 substrates and also modulate the activity of P4503A4 in liver and intestines (Gorski et al., 2004). The Gorski study did not, however, specifically address the 2E1 isoform.

Individual alkylamides present in *E. purpurea* extracts did, indeed, inhibit P450_{2E1}. A total of 11 different alkylamides were detected in the extract used in this study and the structures of several of these are shown in Figure 1. The components were identified based on molecular weights and fragmentation patterns observed with electrospray mass spectrometry as described previously (Cech et al., 2006a). The alkylamides were separated by HPLC (Figure 2) and tested individually for their ability to inhibit P450_{2E1}. Four of these components, undeca-2E,4Z-diene-8,10-dienoic acid isobutylamide, undeca-2Z,4E-diene-8,10-dienoic acid isobutylamide, dodeca-2E,4E,8Z,10Z-tetraenoic acid isobutylamide and dodeca-2E,4E,8Z,10Z-tetraenoic acid isobutylamide (Figure 1) appeared to be most effective at inhibiting the P450 at the concentrations isolated. The isomeric pair of dodeca-tetraenoic acid compounds (peaks 9 + 10) could not be separated, so the mixture of isomers was examined with respect to inhibition of 2E1. Inhibition data for the isolated compounds is shown in Table 2. At a total isobutylamide

concentration of 0.124 mM, the isomeric mixture of compounds found in peak 9 and 10 inhibited 2E1-dependent p-nitrophenol oxidation by 40% or 50% in human liver microsomes (HLM) and expressed 2E1, respectively when 0.020 mM substrate was used. Undeca-2E,4Z-diene-8,10-diynoic acid isobutylamide (Peak 1) also inhibited P450_{2E1}, as did undeca-2Z,4E-diene-8,10-diynoic acid isobutylamide (Peak 2). These two compounds were more potent inhibitors of the P450 than those present in peaks 9+10, however, their concentration in the extract appeared to be somewhat lower. For example, when the 2E,4Z isomer was present in the reaction at 0.030 mM, the oxidation of p-nitrophenol was inhibited by 50 and 53% for human liver microsome and expressed 2E1, respectively, in the presence of 0.020 mM substrate. Under the same conditions, the 2Z, 4E isomer (peak 2) inhibited 2E1 by 40 and 60 % in HLM and expressed P450, respectively, at an alkylamide concentration of 0.025 mM.

To determine the reversibility of the inhibition by the alkylamides, a 10-fold higher concentration of the human liver microsomes were pre-incubated with the inhibitor and NADPH at 30 °C for 20 min. The microsomes that were pretreated in this manner were then diluted 10-fold into a secondary reaction mixture containing the substrate p-nitrophenol and fresh NADPH. Activity of the sample was then compared with the activity of control samples prepared from microsomes pretreated with either the alkylamide alone (no NADPH), or NADPH alone (no alkylamide). The observed activities of all of the pretreated microsomes were identical, indicating a reversible type inhibition by the alkylamide. Several recent studies have shown that cytochrome P450 metabolizes *Echinacea*-derived alkylamides in vitro (Cech et al., 2006b; Woelkart et al., 2005). It has also been reported that one of the isolated alkylamides with a terminal acetylenic group appears to inhibit P450-dependent alkylamide metabolism via a mechanism-based (irreversible) process. We did not see a mechanism-based type inhibition of P450_{2E1}. Based on these observations, it is likely that the metabolism of alkylamides observed in the previous studies was not P450_{2E1}-dependent.

The main alkylamide present in *S. acmella* is spilanthol (structure shown in Figure 1). This compound was isolated using the same procedure utilized for *Echinacea* extracts, and a sample containing highly purified spilanthol was obtained (Figure 3). The identity of this compound as spilanthol was verified by comparison of retention time and molecular weight with literature values (Nagashima et al., 1992). The concentration of alkylamide was estimated using a standard solution of the alkylamide, dodeca-2-(E),4(E)-dienoic acid isobutylamide (Chromadex) and comparing integrated peak areas of spilanthol to those of known amounts of standard. When present at 0.067 mM, spilanthol inhibited P450_{2E1} activity by 55% and 75% in human liver microsome and expressed 2E1 samples, respectively.

Based on these data, it appears that the alkylamides present in *Echinacea* and *Spilanthes* extracts (95% ethanol) are effective inhibitors of P450_{2E1} at concentrations as low as 20 µM. Several recent studies have been published indicating serum alkylamide concentrations reaching the 500 nM range (Mathias et al, 2005b; Gurley et al., 2005). Although these concentrations are roughly 40-fold lower than those used in the current study, serum concentrations may not be an accurate indication of conditions within the ER of the liver. Consequently, the physiological significance of this inhibition is still not clear, and additional research is needed with regard to the bioavailability of these compounds.

Hydrastis canadensis (goldenseal) extracts were also examined with respect to their ability to block cytochrome P450_{2E1} dependent oxidation of p-nitrophenol. Previous studies have shown that certain alkaloid compounds present in goldenseal extracts effectively inhibited several pharmacologically important cytochrome P450 isoforms, including 3A4 and 2D6 (Budzinski et al., 2000). Berberine and hydrastine, the two main alkaloids present in this extract, were especially effective, with K_I values in the mid micromolar range (25–100 µM). In vivo studies have also been performed using *H. canadensis* extracts, and inhibition of P450_{2D6} and 3A4/5

were noted (Gurley et al., 2005). In our studies, goldenseal extracts inhibited the 2E1 enzyme significantly in human liver microsomes. For example, 1 μ L of a 95% ethanol extract in a 0.500 mL reaction inhibited the oxidation of p-nitrophenol in human liver microsomes by >50% using 0.020 mM substrate. On the basis of our chemical analysis of the goldenseal extract, this corresponds to a final alkaloid concentration of 1.7 μ M berberine, 1.2 μ M hydrastine, and 0.10 μ M canadine. Inhibition of P450_{2E1} by goldenseal extracts appeared to be competitive in nature, with a K_I of 0.1% extract. Several of the main alkaloid compounds from *H. canadensis* were then tested individually with regard to their inhibition of 2E1 (Figure 4). The three compounds hydrastine, berberine and canadine were found to significantly inhibit this enzyme with low micromolar K_I values (Table 3). All three inhibitors raised K_m and lowered V_{max} values for the oxidation of p-nitrophenol, indicating a mixed type of inhibition. The K_I values reported are the lower of the two values determined for competitive and non-competitive type inhibition. In particular, berberine, which is the most abundant alkaloid in *H. canadensis*, had a K_I of 18 μ M, indicating even more potent inhibition of this isoform than 3A4 and 2D6, as previously reported (Budzinski et al., 2000). Studies in rat showed that upon oral dosing with 4.38 g/kg body weight of an herbal extract (Huang-Lian-Jie-Du) containing 245 mg berberine and 60 mg palmatine, maximum plasma concentrations of these compounds were 6.2 and 3.1 ng/mL, respectively (Lu et al., 2006). These values are well below the inhibitory concentrations observed in the current study; however, the low plasma levels could be a direct result of rapid first-pass metabolism in the liver, where concentrations may be much higher.

Hydrastine was the most potent of the alkaloid compounds tested at inhibiting P450_{2E1} dependent oxidation of p-nitrophenol. The K_I for this compound was determined to be 2.8 μ M. Although hydrastine is present at lower concentrations than berberine in ethanolic *H. canadensis* extracts, the much lower inhibition constant indicates that it could significantly contribute to the inhibitory properties of *H. canadensis* extract toward cytochrome P450_{2E1} activity.

Green Tea extracts and the associated catechins were also examined for inhibition of human cytochrome P450_{2E1}. Although complete extracts (1.0–5.0 mg/mL) appeared to inhibit the 2E1 enzyme slightly (data not shown), none of the catechins, up to a final concentration of 100 μ M, showed any inhibition toward 2E1.

In conclusion, we have shown that several alkaloid compounds from *H. canadensis* extracts are very potent cytochrome P450_{2E1} inhibitors, with hydrastine being most potent with a K_I of 2.8 μ M, followed by canadine and berberine with K_I values of 17 μ M and 18 μ M, respectively. We have also shown that the alkylamide compounds in *E. purpurea* and *S. acmella* extracts show modest inhibition of P450_{2E1}, with K_I values as low as 20 μ M, and that commercial green tea extract has a minimal effect on P450_{2E1} metabolism.

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References

- Bailey DG, Malcolm J, Arnold O, Spence DJ. Grapefruit juice–drug interactions. *Brit. J. Clin. Pharmacol* 1998;46:101–110. [PubMed: 9723817]

- Brady JF, Li DC, Ishizaki H, Yang CS. Effect of diallyl sulfide on rat liver microsomal nitrosamine metabolism and other monooxygenase activities. *Cancer Res* 1988;48:5937–5940. [PubMed: 3167846]
- Budzinski JW, Foster BC, Vandenhoek S, Arnason JT. An in vitro evaluation of human cytochrome P450 3A4 inhibition by selected commercial herbal extracts and tinctures. *Phytomedicine* 2000;7:273–282. [PubMed: 10969720]
- Castillo T, Koop DR, Kamimura S, Triadafilopoulos G, Tsukamoto H. Role of cytochrome P-450 2E1 in ethanol-, carbon tetrachloride- and iron-dependent microsomal lipid peroxidation. *Hepatology* 1992;16:992–996. [PubMed: 1398507]
- Cech NB, Eleazer MS, Shoffner LT, Crosswhite MR, Davis AC, Mortenson AM. High performance liquid chromatography/electrospray ionization mass spectrometry for simultaneous analysis of alkaloids and caffeic acid derivatives from *Echinacea purpurea* extracts. *J. Chromatogr. A* 2006a; 1103:219–228. [PubMed: 16298376]
- Cech MB, Tutor K, Doty BA, Spelman K, Sasagawa M, Raner GM, Wenner CA. Liver Enzyme-Mediated Oxidation of *Echinacea purpurea* Alkylamides: Production of Novel Metabolites and Changes in Immunomodulatory Activity. *Planta Med* 2006b;72:1372–1377. [PubMed: 17054047]
- Chatterjee P, Franklin MR. Human cytochrome p450 inhibition and metabolic-intermediate complex formation by goldenseal extract and its methylenedioxyphenyl components. *Drug Metab. Dispos* 2003;31:1391–1397. [PubMed: 14570772]
- Ding XX, Koop DR, Crump BL, Coon MJ. Immunochemical identification of cytochrome P-450 isozyme 3a (P-450ALC) in rabbit nasal and kidney microsomes and evidence for differential induction by alcohol. *Mol. Pharmacol* 1986;30:370–378. [PubMed: 3762523]
- Gorsky LD, Coop DR, Coon MJ. On the stoichiometry of the oxidase and monooxygenase reactions catalyzed by liver microsomal cytochrome P-450. Products of oxygen reduction. *J. Biol. Chem* 1984;259:6812–6817. [PubMed: 6725272]
- Gorski JC, Huang SM, Pinto A, Hamman MA, Hilligoss JK, Zaheer NA, Desai M, Miller M, Hall S. The effect of *echinacea* (*Echinacea purpurea* root) on cytochrome P450 activity in vivo. *Clin Pharmacol Ther* 2004;75:89–100. [PubMed: 14749695]
- Guengerich FP, Kim DH. In vitro inhibition of dihydropyridine oxidation and aflatoxin B1 activation in human liver microsomes by naringenin and other flavonoids. *Carcinogenesis* 1990;11:2275–2279. [PubMed: 2265479]
- Guengerich FP, Kim DH, Iwasaki M. Role of human cytochrome P-450 IIE1 in the oxidation of many low molecular weight cancer suspects. *Chem. Res. Toxicol* 1991;4:168–179. [PubMed: 1664256]
- Gurley BJ, Gardner SF, Hubbard MA, Williams DK, Gentry WB, Khan IA, Shah A. In vivo effects of goldenseal, kava kava, black cohosh, and valerian on human P450 1A2, 2D6, 2E1, and 3A4/5 phenotypes. *Clin. Pharmacol. Ther* 2005;77:415–426. [PubMed: 15900287]
- Hammond AH, Fry JR. Involvement of cytochrome P4502E1 in the toxicity of dichloropropanol to rat hepatocyte cultures. *Toxicol* 1997;118:171–179.
- Koul S, Koul JL, Taneja SC, Dhar KL, Jamwal DS, Singh K, Reen RK, Singh J. Structure-activity relationship of piperine and its synthetic analogues for their inhibitory potentials of rat hepatic microsomal constitutive and inducible cytochrome P450 activities. *Bioorg. Med. Chem* 2000;8:251–268. [PubMed: 10968285]
- Larson JR, Coon MJ, Porter TD. Purification and properties of a shortened form of cytochrome P-450 2E1: deletion of the NH2-terminal membrane-insertion signal peptide does not alter the catalytic activities. *Proc. Natl. Acad. Sci. U. S. A* 1991;88:9141–9145. [PubMed: 1656462]
- Lee M-J, Prabhu S, Meng X, Li C, Yang CS. An improved method for determining green and black tea polyphenols in biomatrices by high-performance liquid chromatography with coulometric array detection. *Anal. Biochem* 2000;279:164–169. [PubMed: 10706785]
- Lu T, Liang Y, Song J, Xie L, Wang GJ, Liu XD. Simultaneous determination of berberine and palmatine in rat plasma by HPLC-ESI-MS after oral administration of traditional Chinese medicinal preparation Huang-Lian-Jie-Du decoction and the pharmacokinetic application of the method. *J. Pharm. Biomed. Anal* 2006;40:1218–1224. [PubMed: 16293389]

- Matthias A, Gillam EMJ, Penman KG, Matovic NJ, Bone KM, De Voss JJ, Lehmann RP. Cytochrome P450 enzyme-mediated degradation of Echinacea alkylamides in human liver microsomes. *Chemico-Biol. Interact* 2005a;155:62–70.
- Matthias A, Addison RS, Penman KG, Dickinson RG, Bone KM, Lehmann RP. Echinacea alkamide disposition and pharmacokinetics in humans after tablet ingestion. *Life Sciences* 2005b;77:2018–2029. [PubMed: 15919096]
- Nagashima M, Nakatani N. LC -MS analysis and structure determination of pungent alkamides from *Spilanthes acmella* L. flowers. *Lebensmittel-Wissenschaft und -Technologie* 1992;25:417–421.
- Sasagawa M, Cech NB, Gray DE, Elmer GW, Wenner CA. Echinacea alkylamides inhibit interleukin-2 production by Jurkat T cells. *Int. Immunopharmacol* 2006;6:1214–1221. [PubMed: 16714226]
- Subehan UT, Kadota S, Tezuka Y. Mechanism-based inhibition of human liver microsomal cytochrome P450 2D6 (CYP2D6) by alkamides of *Piper nigrum*. *Planta Med* 2006;72:527–532. [PubMed: 16808005]
- Subramanian U, Ahmed AE. Intestinal toxicity of acrylonitrile: in vitro metabolism by intestinal cytochrome P450 2E1. *Toxicol. Appl. Pharmacol* 1995;135:1–8. [PubMed: 7482528]
- Wentworth JM, Agostini M, Love J, Schwabe JW, Chatterjee VK. St.John's Wart, an herbal antidepressant, activates the steroid X receptor. *J. Endocrineol* 2000;166:R11–R16.
- Woelkart K, Koidl C, Grisold A, Gangemi JD, Turner RB, Marth E, Bauer R. Bioavailability and pharmacokinetics of Alkamides from the roots of *Echinacea angustifolia* in humans. *J. Clin. Pharmacol* 2005;45:683–689. [PubMed: 15901750]
- Xu Y, Leo MA, Lieber CS. Lycopene attenuates arachidonic acid toxicity in HepG2 cells overexpressing CYP2E1. *Biochem. Biophys. Res. Commun* 2003;303:745–750. [PubMed: 12670473]
- Yale SH, Glurich I. Analysis of the inhibitory potential of *Ginkgo biloba*, *Echinacea purpurea*, and *Serenoa repens* on the metabolic activity of cytochrome P450 3A4, 2D6, and 2C9. *J. Altern. Complement Med* 2005;11:433–439. [PubMed: 15992226]
- Yang S-P, Medling T, Raner GM. Cytochrome P450 Expression and Activities in the Rat, Rabbit and Bovine Tongue. *Comp. Biochem. Physiol. C* 2003;136:297–308.
- Yang S-P, Raner GM. Cytochrome P450 Expression, Induction and Activities in Human Tongue Cells and their Modulation by Green Tea Extract. *Toxicol. Appl. Pharmacol* 2005;202:140–150. [PubMed: 15629189]

Figure 1. Chemical structures of major alkylamide components from *Echinacea purpurea* and *Spilanthes acmella*. Compounds 1, 2, 9 and 10 were isolated from *E. purpurea* (95 % ethanol extract), whereas spilanthol was isolated from an *S. acmella* extract (95 % ethanol).

Figure 2.

HPLC chromatogram showing the relative abundance of 11 different alkylamides from a 100 % ethanol extract from *Echinacea purpurea*. The molecular weight of each of the components was determined by isolating the fractions containing that peak and subjecting them to ESI-MS analysis. The molecular weights of the *protonated molecular ion* that corresponds to each peak is indicated on the chromatogram. It should be noted that these molecular weights are one larger than the weights shown in Figure 1 because of the additional mass of the proton.

Figure 3. HPLA chromatograms showing alkylamide content of raw (100 % ethanol) *Spilanthes acmella* extract and a sample of spilanthol purified from the crude extract. Spilanthol is the primary alkylamide component of the raw extract.

Figure 4. Michaelis-Menton plots showing the inhibition of cytochrome P450_{2E1}-dependent oxidation of p-nitrophenol by (+)hydrastine, (●) canadine, and (▲) berberine, in pooled human liver microsomes. Activities are compared to the control reaction (♦) in which no inhibitor was present.

Table 1

Inhibition of human P450_{2E1} by various extracts (the total volume for each reaction was 0.500 mL).

Extract	[p-nitrophenol]	Enzyme	% inhibition
Echinacea extracted in 95% ethanol (2 µL)	0.020 mM	HLM	27 %
		Expressed 2E1	29 %
Echinacea extract in 33 % ethanol (2µL)	0.020 mM	HLM	0 %
		Expressed 2E1	0 %
Spilanthes extracted in 95 % ethanol (40 µL)	0.020 mM	HLM	40 %
		Expressed 2E1	60 %

Table 2

Inhibition of human P450_{2E1} by isolated isobutylamides from *E. purpurea* and *S. acmella* (the total reaction volume was 0.500 mL).

Isobutylamide from figure 1	[p-nitrophenol]	Enzyme	% inhibition
I @ 0.030 mM	0.020 mM	HLM	50 %
		Expressed 2E1	53 %
II @ 0.025 mM	0.020 mM	HLM	40 %
		Expressed 2E1	62 %
IX + X @ 0.125 mM	0.020 mM	HLM	40 %
		Expressed 2E1	53 %
Spilanthol @ 0.067 mM	0.020 mM	HLM	55%
		Expressed 2E1	75 %

Table 3

Inhibition of P450_{2E1} activity in human liver microsomes by goldenseal extract and individual alkaloid components. Final reaction volumes were 0.500 mL.

Inhibitor	[p-nitrophenol]	% inhibition	K _I
Goldenseal extract (1 µL of 50 % ethanol extract)	0.020 mM	55%	0.1 % extract
Berberine (4.0 µM)	0.020 mM	11 %	18 µM
Canadine (4.0 µM)	0.020 mM	8 %	17 µM
Hydrastine (4.0 µM)	0.020 mM	64 %	2.8 µM