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# The effects of commercial preparations of herbal supplements commonly used by women on the biotransformation of fluorogenic substrates by human cytochromes P450

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**The effects of commercial preparations of herbal supplements commonly used by women on the biotransformation of fluorogenic substrates by human cytochromes P450**

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3 **The effects of commercial preparations of herbal supplements commonly used by**  
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5 **women on the biotransformation of fluorogenic substrates by human**  
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7 **cytochromes P450**  
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**ABSTRACT**

We set out to determine the potential for commercially available preparations of black cohosh (*Actaea racemosa*), chaste tree berry (*Vitex agnus-castus*), crampbark (*Viburnum opulus*) and false unicorn (*Chamaelirium luteum*) to inhibit the major human drug metabolizing enzymes CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 as well as CYP1A1 which activates some carcinogens. *In vitro* microplate-based assays using cDNA-expressed CYP450 isoforms and fluorogenic substrates were used. Components of the commercial herbal preparations interfered with the assays and limited the concentration ranges that could be tested. Nevertheless, the fluorogenic assays were robust, reproducible and easy to perform and thus are still useful for initial screening for potential herb/drug interactions. None of the preparations affected CYPs 1A1 or 2C9 at the concentrations tested but all preparations inhibited some of the enzymes with potencies around 1 µg/mL. The three most potent interactions were: chaste tree berry and CYP2C19 (IC<sub>50</sub> 0.22 µg/mL); chaste tree berry and CYP3A4 (IC<sub>50</sub> 0.3 µg/mL); black cohosh and CYP2C19 (IC<sub>50</sub> 0.37 µg/mL). Thus, we have successfully identified the potential for the commercial herbal preparations to inhibit human drug metabolizing enzymes. Whether this potential translates into clinically significant herb/drug interactions can only be confirmed by appropriate *in vivo* studies.

## Introduction

Herbal products are gaining in popularity in large part because consumers believe that they are “natural”, safer, more effective, have fewer side effects, and can be obtained at a lower cost than conventional therapies (Elvin-Lewis, 2001). Consequently, herbal products are widely used by women to alleviate discomfort associated with menstruation, pregnancy/labour, premenstrual syndrome and menopause (Beal, 1998). Herbals commonly used for these reproductive complaints include black cohosh (*Actaea racemosa*), chaste tree berry (*Vitex agnus-castus*), crampbark (*Viburnum opulus*), and false unicorn (*Chamaelirium luteum*) (Beal, 1998; Roemheld-Hamm, 2005; Ross, 2007)

Natural products are often thought to be safe, yet some herbal preparations contain constituents that inhibit cytochromes P450 (CYP450) (Fugh-Berman and Ernst, 2001). Since many women take both herbals and prescription medications simultaneously (Refuerzo *et al*, 2005) there is a potential for interactions to occur (Fugh-Berman and Ernst, 2001). However, there is no predictive pharmacokinetic database that would assist users and prescribers in avoiding potential interactions. This is in stark contrast to the development of pharmaceuticals where predictive pharmacokinetics plays a major role in the progression from lead compound to candidate drug (Cohen *et al*, 2003). Other important differences between pharmaceuticals and herbals are: i) that the former normally have only one active chemical entity whereas herbals – including those in this study – often have multiple components (Gödecke *et al*, 2009), and those components that are pharmacologically active are not necessarily the ones that are pharmacokinetically active; ii) that the

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3 relative amounts of various constituents of herbals can vary markedly from  
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5 preparation to preparation (Gödtel-Armbrust *et al*, 2007).  
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10 The specific aim of this study was to establish predictive CYP450 interaction  
11 kinetics for specific commercial preparations of black cohosh root, chaste tree berry,  
12 crampbark and false unicorn that are readily available to consumers in Hamilton,  
13 Ontario. To achieve this we examined the ability of these herbal preparations to  
14 inhibit the biotransformation of fluorogenic substrates by the major human drug  
15 metabolizing enzymes CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 which  
16 account for 5%, 10%, 15%, 20-30%, and 40-45% of total drug biotransformations,  
17 respectively (Ingelman-Sundberg, 2004). We also investigated their actions on human  
18 CYP1A1, which is implicated in both the activation and detoxification of a number of  
19 carcinogens (Androutsopoulos *et al*, 2009). Since there is some controversy about the  
20 appropriateness of various methods for screening CYP activity (Strandell *et al*, 2004;  
21 Unger and Frank, 2004), a more general aim was to assess the usefulness of the rapid  
22 screening of herbals against human cytochromes P450 using fluorogenic substrates as  
23 an efficient way to acquire information on potential interactions.  
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## Materials and Methods

### *Materials*

All assay reagents were purchased from Sigma-Aldrich (Oakville, ON, Canada). Recombinant human cytochrome P450 enzymes (CYP450) were obtained from BD Biosciences (Mississauga, ON, Canada). All substrates and fluorescent product standards were obtained from BD Gentest (Woburn, MA, USA) except for the Vivid<sup>®</sup> Red CYP2C9 (Vivid Red) substrate from Invitrogen (Burlington, ON, Canada). Solvents were of analytic or high-performance liquid chromatography (HPLC) grade. Commercial liquid (ethanolic) extracts of black cohosh and chaste tree berry (Gaia Herbs, Brevard, NC, USA) and crampbark and false unicorn (St Francis Farms, Cobermere, ON, Canada) were obtained from local retailers.

### *Preparation of Herbal Products*

Commercial extracts of the herbal preparations were diluted in assay buffer so that the final concentration of ethanol in the assays was 1%.

### *Cytochrome P450 Assay*

The velocity of biotransformation of 7-ethoxyresorufin (ERES) by CYP1A1 (0.2 pmol/well), 3-cyano-7-ethoxycoumarin (CEC) by CYP1A2 & CYP2C19 (0.5 and 2 pmol/well, respectively), Vivid Red by CYP2C9 (6.8 pmol/well), 3-[2-(N,N-diethyl-N-methylammonium)-ethyl]-7-methoxy-4-methylcoumarin (AMMC) by CYP2D6 (5 pmol/well) and 7-benzyloxyquinoline (BQ) by CYP3A4 (1 pmol/well) were determined by fluorometric assays using the general principles as described (Crespi *et al*, 1997; Marks *et al*, 2004). Reactions were carried out at 37°C in black 96-well

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3 plates (Costar) with a final volume of 200  $\mu$ L containing (final concentrations):  
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6 potassium phosphate buffer pH 7.4 (0.15M), NADP (330  $\mu$ M), glucose-6-phosphate  
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8 (830  $\mu$ M),  $MgCl_2$  (850  $\mu$ M), glucose-6-phosphate dehydrogenase (70-75 mU/well),  
9  
10 substrate (variable), and recombinant human CYP450s. Assays were initiated by the  
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12 addition of enzyme. Formation of product was monitored every 40s for 15 minutes  
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14 using a SpectraMax Gemini XS reader (Molecular Devices) except in the case for  
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16 CYP3A4 which was monitored for 7 minutes due to the high velocity of the reaction  
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18 under the conditions used. Reaction velocities were calculated using the instrument's  
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20 software (Softmax Pro). In all cases the fluorescence intensity was calibrated with  
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22 authentic product and the duration of the incubation and the CYP450 concentration  
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24 were in the linear range of metabolite formation. In experiments to examine the ability  
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26 of standard compounds and herbal preparations to inhibit CYP450 activity, wells  
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28 contained fixed concentrations of substrate (Table 1), the putative inhibitors, and the  
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30 appropriate vehicle which was also present in control wells.  
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### 39 *Detection of autofluorescence and fluorescence quenching*

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41 Increasing concentrations of herbal preparations were diluted in phosphate buffer or  
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43 1% ethanol and incubated with fixed concentrations of fluorescent products. The  
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45 concentrations used were those that would be present in control wells of inhibition  
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47 experiments at the end of the incubation period. They were determined from kinetic  
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49 experiments and were as follows: resorufin (CYP1A1 and 2C9), 0.025  $\mu$ M and 0.05  
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51  $\mu$ M; 3-cyano-7-hydroxycoumarin (CYP1A2 and 2C19) 0.2 $\mu$ M and 0.05 $\mu$ M; 3-[2-  
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53 (N,N-Diethyl-N-methylammonium)ethyl]-7-hydroxy-4-methylcoumarin (CYP2D6),  
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55 0.1  $\mu$ M; 7-hydroxyquinoline (CYP3A4), 2.25  $\mu$ M. Incubations were carried out at  
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60 37°C in black 96-well plates (Costar) with a final volume of 200  $\mu$ L. Single



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3 fluorimetric measurements were made using the SpectraMax Gemini XS reader  
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5 (Molecular Devices) with the appropriate excitation and emission wavelengths.  
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### 10 *Data Analysis*

11  
12 Kinetic parameters for each of the enzymes were determined by fitting plots of the  
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14 reaction velocity versus substrate concentration to different kinetic models by  
15  
16 nonlinear least-squares regression. The simplest model was the Michaelis-Menten  
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18 model given by the equation:  
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$$22 \quad V = (V_{\max} * [S]) / (K_m + [S]) \quad (1)$$

23  
24 where V is the reaction velocity at substrate concentration [S],  $V_{\max}$  is the maximum  
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26 velocity of the reaction and  $K_m$  is the Michaelis-Menten constant.  
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32 In some cases, the Hill model which can be described by equation 2 was used:  
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$$36 \quad V = (V_{\max} * [S]^n) / (K_m^n + [S]^n) \quad (2)$$

37  
38 where  $n$  is the Hill coefficient.  
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41 In cases where a downturn in the enzyme kinetics at high substrate  
42  
43 concentrations suggested the possibility of substrate inhibition, this was followed by  
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45 fitting the data to equation (3), adopted from Kenworthy *et al* (2001) based on the  
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47 principles defined by Segel (1975), which assumes that the substrate binds with equal  
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49 affinity to two sites on the enzyme and allows for interaction between the two sites:  
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$$53 \quad V = V_{\max} * (([S] / K_m) + (\beta * [S]^2 / K_m^2)) / (1 + (2[S] / K_m) + ([S]^2 / K_m^2)) \quad (3)$$

54  
55 where  $\beta$  reflects the degree to which the two occupied sites interact to alter the  
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57 effective catalytic rate constant (when  $\beta = 1$  there is no change in the rate of product  
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formation). The applicability of increasingly complex models over simple models was assessed using the F-test (Motulsky and Ransnas, 1987) at  $P < 0.05$ .

The effects of putative inhibitors were expressed as a percentage of control according to the following equation:

$$E = ((V_c - B) / (C - B)) * 100 \quad (4)$$

where E represents the percentage change with respect to control,  $V_c$  is the reaction velocity in the presence of putative inhibitor, B is the reaction velocity in the absence of enzyme and C is the reaction velocity in the absence of putative inhibitor.

Inhibitory effects were subsequently assessed using a nonlinear least-squares fitting to the simple competitive binding equation:

$$E = 100 - (100 / (1 + (10^{(-pIC_{50} - \log[X])}))) \quad (5)$$

where [X] is the concentration of the putative inhibitor and  $pIC_{50}$  is the negative log of the concentration that inhibits enzyme activity by 50%. For herbals the  $pIC_{50}$  is in units of g/mL. For standard compounds, the molar  $pIC_{50}$  values were converted to  $pK_i$  (molar inhibition constant) values according to equation (6) adapted from Cheng & Prusoff (1973) on the assumption that the nature of inhibition is independent of the kinetic model:

$$pK_i = -\log_{10}((10^{-pIC_{50}}) / (1 + ([S]/K_m)))$$

(6)

When data were tabulated, the  $pIC_{50}$  and  $pK_i$  values were converted to  $IC_{50}$  ( $\mu\text{g/mL}$ ) and  $K_i$  ( $\mu\text{M}$ ), respectively, for ease of interpretation.  $IC_{50}$  values were also compared to the concentrations ( $\mu\text{g/mL}$ ) that would be attained from single recommended doses

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3 of the herbals at different volumes of distribution, assuming 100% bioavailability.  
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6 The volumes of distribution used were 3, 15 and 40L (to represent plasma volume, the  
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8 volume of extracellular water, and total body water, respectively). Single dose values  
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10 were as follows: Black cohosh, 500 mg; chaste tree berry, 333 mg; crampbark, 250  
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12 mg; false unicorn, 200 mg.  
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18 All data fitting was performed in Microsoft Excel using the Solver function.

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20 The results of all experiments are expressed as mean  $\pm$  S.E.M. of three to four  
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22 experiments performed in triplicate.  
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## Results

Biotransformation of fluorogenic substrates by the cytochrome P450 enzymes tested in the present study was linear with time as shown in the representative graphs (Figs 1A, 1C). Kinetic plots of reaction velocity versus substrate concentration for CYP1A1, CYP2C9 and CYP2D6 were best fit by simple Michaelis-Menten kinetics (eq 1), whereas CYP1A2 (Fig 1B) and CYP2C19 followed complex kinetics described by the Hill model (eq 2). The kinetics for CYP3A4 (Fig 1D) followed the substrate inhibition model described by equation (3). Table 2 summarizes the kinetic parameters of the cytochrome P450 enzymes and the effects of standard inhibitors are given in Table 1.

All herbal preparations produced some degree of fluorescence quenching or autofluorescence at some wavelengths when used at high concentrations. This often reduced the maximum concentrations that could be used in the assays. By limiting the maximum concentrations used to those producing no more than a 10% change in the fluorescence intensity of the amount of product generated under standard conditions, we were still able to estimate the inhibitory potential of the herbal preparations. The maximum concentrations for each herbal used in each assay are listed in Table 3.

Concentration-dependent inhibition of CYP450 activity by chaste tree berry extract is shown in Fig. 2. The effects of all the herbal preparations on the human CYP450 enzymes are summarized in Table 4. CYP1A2, CYP2C19, CYP2D6 and CYP3A4 were each inhibited by at least one of the herbal preparations, whereas CYP1A1 and CYP2C9 were unaffected. For preparations that showed activity, the

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3 IC<sub>50</sub> values ranged from as low as 0.22 µg/mL to as high as 9.7 µg/mL, in all these  
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6 cases, concentrations higher than those required to produce 50% inhibition of CYP  
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8 activity would be attained from a single dose of herbal even if the active compound(s)  
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10 were distributed in the total body water volume, assuming 100% bioavailability.  
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For Peer Review

## Discussion

We chose to use fluorimetric, microplate-based assays because they are homogeneous, sensitive, easy to perform, and have a high throughput that would be ideal for initial screening of large libraries of commercial herbal products for potential interactions with cytochromes P450 (Crespi *et al*, 1997; Ghosal *et al*, 2003; Strandell *et al*, 2004). The kinetic parameters that we determined (Table 2) are in good agreement with previously published data (Ghosal *et al*, 2003; Marks *et al*, 2004; Stresser *et al*, 2000), some minor differences are likely due to different experimental conditions. We detected atypical kinetics for CYP1A2, CYP2C19, and CYP3A4 (Table 2). Complex allosteric interactions are fairly common among cytochromes P450 (Houston and Kenworthy, 2000), but are not observed consistently (Ghosal *et al*, 2003; Stresser *et al*, 2000). We validated the assays by showing that standard inhibitors of each respective CYP isoform acted with an appropriate potency (Table 1). Again, there is good agreement between our data and those of others using quite diverse methods (Ghosal *et al*, 2003; Marks *et al*, 2004; Shimada and Guengerich, 2006; Unger and Frank, 2004).

We began this study with the assumption that the benefits of fluorimetric, microplate-based assays would outweigh those of alternate methods such as liquid chromatography/mass spectrometry (Unger and Frank, 2004) which require complex instrumentation, dedicated personnel and additional sample extraction procedures. One notable disadvantage of fluorogenic substrates is that results obtained do not always agree with those from conventional substrates. For instance there was a large discrepancy between the observed interaction of compounds with CYP3A4 when the fluorogenic probe Vivid Red was used as substrate as opposed to when the

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3 conventional substrate testosterone was used, on the other hand, there was good  
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5 correlation for CYP2C19 between results obtained with the fluorogenic substrate  
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8 Vivid Blue and the conventional substrate S(+)-mephenytoin (Cohen *et al*, 2003).  
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10 This phenomenon puts some constraints upon the interpretation of the data (Cohen *et*  
11 *al*, 2003). However, there is also lack of agreement among conventional substrates,  
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13 and a general principle that the choice of the probe substrate has a significant impact  
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15 on the drug interaction profile of a given agent has emerged (Foti and Wahlstrom,  
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17 2008). Clearly, with a task as large as the initial screening of herbals for their  
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19 interactions with CYP450s, the use of a library of substrates for each isoform is  
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21 impractical. Cohen *et al* (2003) support the continued use of fluorogenic substrates  
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23 for the initial identification of the potential of new chemical entities to interact with  
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25 CYP450s, provided that follow-up studies are carried out on drug candidates with  
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27 conventional probes. Similar principles could be adopted for herbals with follow-up  
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29 studies being performed on the more potent interactions.  
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39 We compared the inhibitory potencies of the herbals to the concentrations that  
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41 would be attained from single recommended doses of each preparation at three  
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43 different volumes of distribution which corresponded to plasma volume, the volume  
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45 of extracellular water and total body water by assuming 100% bioavailability of the  
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47 active principle (Table 4). For black cohosh against CYPs 2D6 and 1A2  
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49 concentrations from a single dose distributed in total body water were approximately  
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51 7 and 35 times higher than the *in vitro* IC<sub>50</sub> values, respectively. Thus, the potencies  
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53 of black cohosh against these two isoforms seem sufficiently high to merit concern,  
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55 yet *in vivo* data argue against this (Gurley *et al*, 2005; Gurley *et al*, 2008).  
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3 Results of this study did identify some potent inhibition of CYP450 activity  
4 (Table 4) by commercially available herbal preparations. Black cohosh exhibited  
5 inhibitory activity against CYP 1A2, 2C19 and 2D6. Although some cycloartanoid  
6 triterpene glycoside components of black cohosh have weak inhibitory activity against  
7 CYP3A4 (Tsukamoto *et al*, 2005), we found no effect of the commercial extract at the  
8 low concentrations we were able to test, and this is in agreement with *in vivo* data  
9 suggesting no clinically relevant interactions between black cohosh and CYP3A4  
10 (Gurley *et al*, 2006). Similar *in vivo* experiments discount the clinical significance of  
11 the inhibition of CYP2D6 by black cohosh (Gurley *et al*, 2008) which was significant  
12 in our assays but was the lowest activity that we measured (Table 4). *In vivo* studies  
13 also do not support any clinically significant interactions between black cohosh and  
14 CYP1A2 (Gurley *et al*, 2005) whereas we found the inhibition to be quite potent  
15 (Table 4). However, the most potent inhibitory effect of black cohosh was against  
16 CYP2C19 (Table 4) for which no *in vivo* data are available at present. While the  
17 discrepancies might be explained by differences in the herbal preparations used in the  
18 *in vivo* studies compared to ours, a more likely explanation is that the active inhibitory  
19 components are poorly bioavailable. The majority of evidence (summarised by Unger  
20 and Frank, 2004), suggests that polyphenolic constituents of herbals have a very low  
21 oral bioavailability, while other components are reported to have high bioavailability  
22 (see Strandell *et al*, 2004).  
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53 Adverse events following the consumption of chaste tree berry are usually  
54 mild and reversible (Daniele *et al*, 2005; Dugoua *et al*, 2008) with no drug  
55 interactions identified by systematic reviews of the literature. However, neither *in*  
56 *vitro* nor *in vivo* pharmacokinetic studies with this herb have been reported to date.  
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3 We found that chaste tree berry extract inhibited CYPs 1A2, 2C19, 2D6 and 3A4 at  
4 concentrations in the  $\mu\text{g/mL}$  range or lower. In comparison to predicted  
5 concentrations *in vivo*, effects on CYPs 2C19 and 3A4 were particularly potent (Table  
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There are no prior studies on the pharmacokinetics of crampbark and false unicorn. Both were potent inhibitors with  $\text{IC}_{50\text{s}}$  close to  $1 \mu\text{g/mL}$ ; crampbark of CYPs 1A2 and 2C19 and false unicorn of CYPs 2D6 and 3A4 (Table 4).

Because of some necessary assumptions about pharmacokinetic properties of test substances and the nature of the assays themselves, CYP450 inhibition kinetics cannot be relied upon to accurately predict herb/drug interactions. This principle is aptly demonstrated in the case of black cohosh discussed above where its high *in vitro* potency against CYPs 2D6 and 1A2 does not lead to significant clinical effects. However, the cost of performing *in vivo* experiments on all herbal preparations – let alone the enormity of the task – would be prohibitive. Therefore a more practical approach is to accept the lower predictive power of CYP450 inhibition kinetics alone, prioritise preparations according to their inhibitory potency and their frequency of use and then proceed directly to *in vivo* experiments. Although the methods we have described have some limitations brought about by inherent fluorescence properties of some herbals, they provide a relatively fast and efficient means of obtaining the necessary preliminary data on large numbers of preparations simultaneously.

In summary, the use of fluorogenic substrates to screen for the effects of herbal preparations on the activity of human CYP450s is limited by interference from

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3 components of the herbals themselves. Nevertheless, we have been able to  
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5 demonstrate that several commercial preparations that are used by women are potent  
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7 (IC<sub>50</sub>  $\simeq$  1  $\mu$ g/mL) inhibitors of CYP450 activity. Caution in their concomitant use  
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9 with pharmaceuticals that are biotransformed by these same enzymes is advised but *in*  
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13 *vivo* research to investigate the clinical significance of the interactions is necessary.  
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For Peer Review

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Table 1. Assay conditions for the effects of putative inhibitors on the biotransformation of fluorogenic substrates by human cytochrome P450 enzymes and the effects of standard compounds

Enzyme	Substrate	Substrate concentration ( $\mu\text{M}$ )	Inhibitor	$K_i$ ( $\mu\text{M}$ )	$pK_i$
CYP1A1	ERES	0.5	Benzo[a]pyrene	0.05	$7.27 \pm 0.06$
CYP1A2	CEC	5	Furafylline	2.9	$5.4 \pm 0.3$
CYP2C19	CEC	25	Tranlycypromine	2	$5.70 \pm 0.03$
CYP2C9	Vivid Red	5	Sulfaphenazole	0.1	$6.97 \pm 0.04$
CYP2D6	AMMC	2	Quinidine	0.005	$8.3 \pm 0.1$
CYP3A4	BQ	50	Ketoconazole	0.03	$7.52 \pm 0.02$

Values for  $pK_i$  are means  $\pm$  SEM from 3-4 experiments performed in duplicate. The  $K_i$  values were derived from the  $pK_i$  values to facilitate interpretation of the data.

Table 2. Kinetic parameters for the biotransformation of fluorogenic substrates by human cytochrome P450 enzymes

Enzyme	Substrate	Model	$K_m$ ( $\mu\text{M}$ )	$V_{\text{max}}$ (pmol/min/pmol)	Coeff
CYP1A1	ERES	M-M	$0.2 \pm 0.2$	$1.9 \pm 0.2$	-
CYP1A2	CEC	Hill	$2.7 \pm 0.1$	$6.8 \pm 0.4$	$0.7 \pm 0.1$
CYP2C19	CEC	Hill	$21 \pm 1$	$0.2 \pm 0.03$	$1.9 \pm 0.3$
CYP2C9	Vivid Red	M-M	$1.3 \pm 0.1$	$0.07 \pm 0.01$	-
CYP2D6	AMMC	M-M	$1.4 \pm 0.01$	$0.8 \pm 0.1$	-
CYP3A4	BQ	SI	135	111	0.06

Data are presented as means  $\pm$  SEM from 3-4 experiments, except for CYP3A4 where they are the means of two experiments. All experiments were performed in triplicate.

MM, Michaelis-Menten; SI, substrate inhibition; Coeff, coefficient

Table 3. Highest concentrations of herbal preparations used in human cytochrome P450 assays

Enzyme	CYP1A1	CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4	
Substrate	ERES	CEC	CEC	Vivid Red	AMMC	BQ	Predicted <i>in vivo</i> *
Black Cohosh	5	5	15	5	50	0.15	33
Chaste Tree Berry	3.3	33	10	6	33	10	22
Crampbark	2.5	7.5	75	2.5	0.25	0.025	17
False Unicorn	0.6	0.6	6	3.3	20	2	13

Units are  $\mu\text{g}/\text{mL}$ . \* From a single dose as described in Methods assuming 100% bioavailability and distribution in an extracellular volume of 15L.

Table 4. Effects of herbal preparations on the biotransformation of fluorogenic substrates by human cytochromes P450

Herbal	CYP1A2	CYP2C19	CYP2D6	CYP3A4	Single dose concentration ( $\mu\text{g/mL}$ ) at different volumes of distribution
	$\text{IC}_{50}$ ( $\mu\text{g/mL}$ )	$\text{IC}_{50}$ ( $\mu\text{g/mL}$ )	$\text{IC}_{50}$ ( $\mu\text{g/mL}$ )	$\text{IC}_{50}$ ( $\mu\text{g/mL}$ )	3L
	$p\text{IC}_{50}$ (g/mL)	$p\text{IC}_{50}$ (g/mL)	$p\text{IC}_{50}$ (g/mL)	$p\text{IC}_{50}$ (g/mL)	15L
					40L
Black cohosh	1.8	0.37	9.7	-	167
	$5.7 \pm 0.1$	$6.44 \pm 0.03$	$5.01 \pm 0.06$		33
Chaste tree berry	3.8	0.22	2.9	0.3	111
	$5.4 \pm 0.1$	$6.66 \pm 0.05$	$5.5 \pm 0.1$	$6.52 \pm 0.08$	22
Cramp bark	0.94	1.1	-	-	8
	$6.03 \pm 0.05$	$5.97 \pm 0.05$			83
False unicorn	-	-	0.8	0.5	17
			$6.1 \pm 0.1$	$6.30 \pm 0.05$	6
					67
					13
					5

The  $p\text{IC}_{50}$  values are expressed as means  $\pm$  SEM from 3-6 experiments performed in duplicate.  $\text{IC}_{50}$  values and the concentrations attained from single recommended doses at different volumes of distribution were determined as described in Methods. Where no values are given,  $\text{IC}_{50}$  values were greater than the maximum concentrations used in the assays (Table 3). Neither CYP1A1 nor CYP2C6 were affected by any of the herbals tested at the concentrations shown in Table 3.

**Figure Legends****Figure 1.**

Effect of substrate concentration on the biotransformation of fluorogenic substrates by human cytochromes P450 1A2 (A and B) and 3A4 (C and D). (A) The time course of formation of CHC from CEC by CYP1A2 was determined at ten concentrations of substrate by measuring the fluorescence intensity every 40 s over a 15 min period as described in Methods. Lines are linear least-squares regressions. (B) Concentration / velocity plot of the data in (A). (C) The time course of formation of 7-HQ from 7-BQ by CYP3A4 was determined at ten concentrations of substrate by measuring the fluorescence intensity every 40 s over a 7 min period as described in Methods. Lines are linear least-squares regressions. (D) Concentration / velocity plot of the data in (C). The line shows the fit of the data to equation (3). Each point represents the mean  $\pm$  SEM of triplicate observations in a single experiment.

**Figure 2.**

The effects of increasing concentrations of chaste tree berry on the biotransformation of (A) CEC by CYP1A2, (B) CEC by CYP2C19, (C) AMMC by CYP2D6 and (D) 7-BQ by CYP3A4 as described in methods. The line of best fit was determined from the data by equation (5). Points are means  $\pm$  SEM of duplicates in a single experiment.

Figure 1.

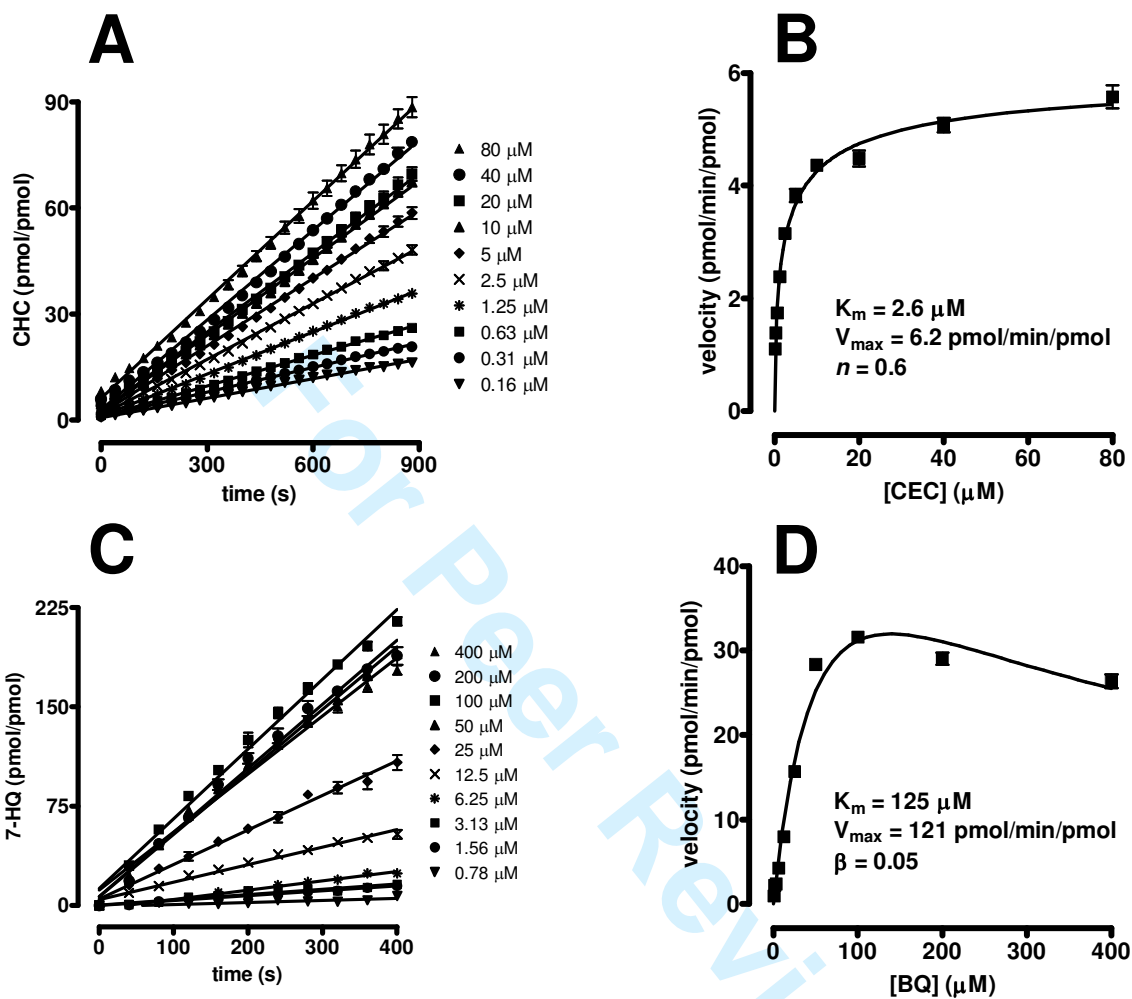
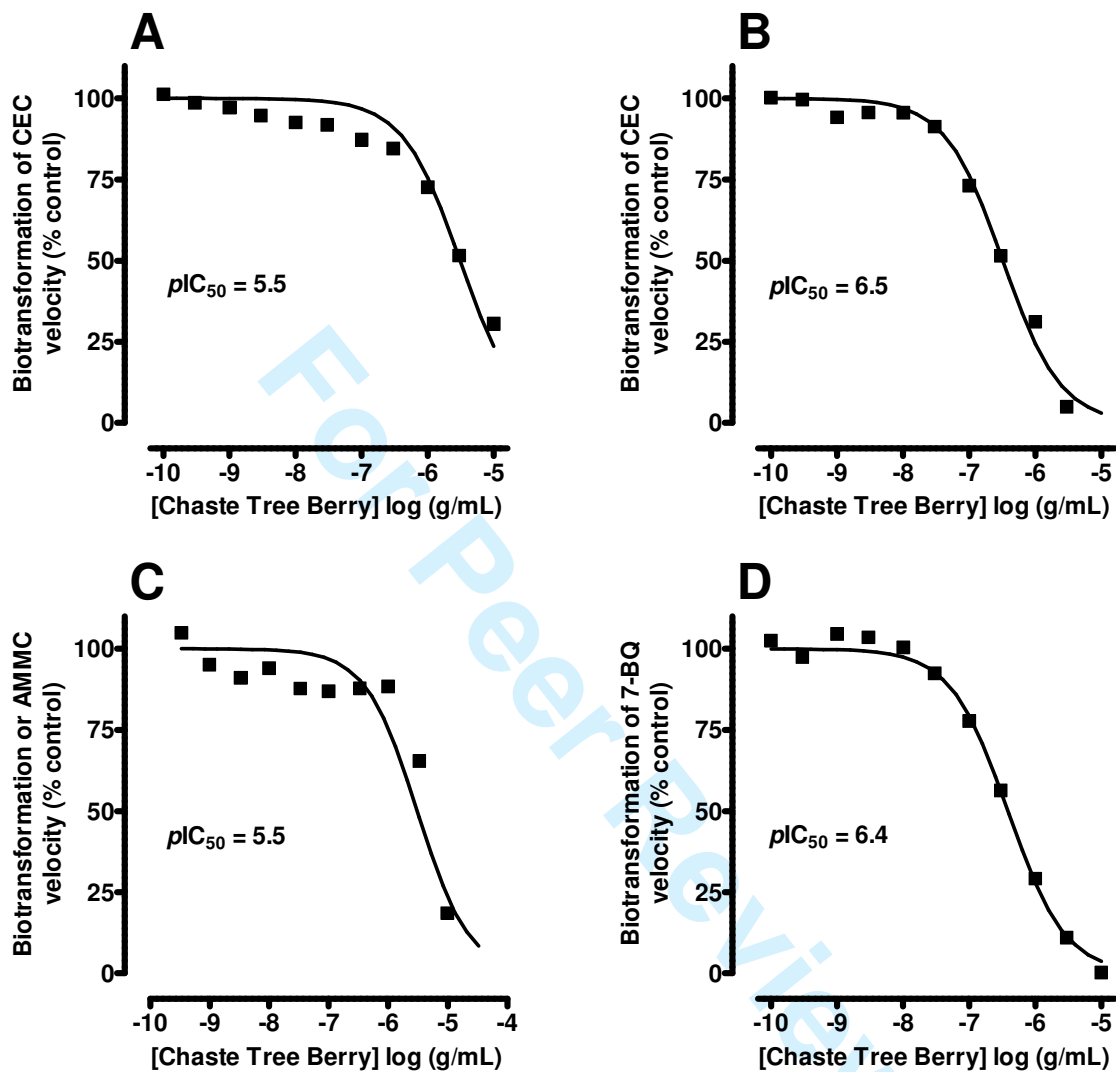


Figure 2.



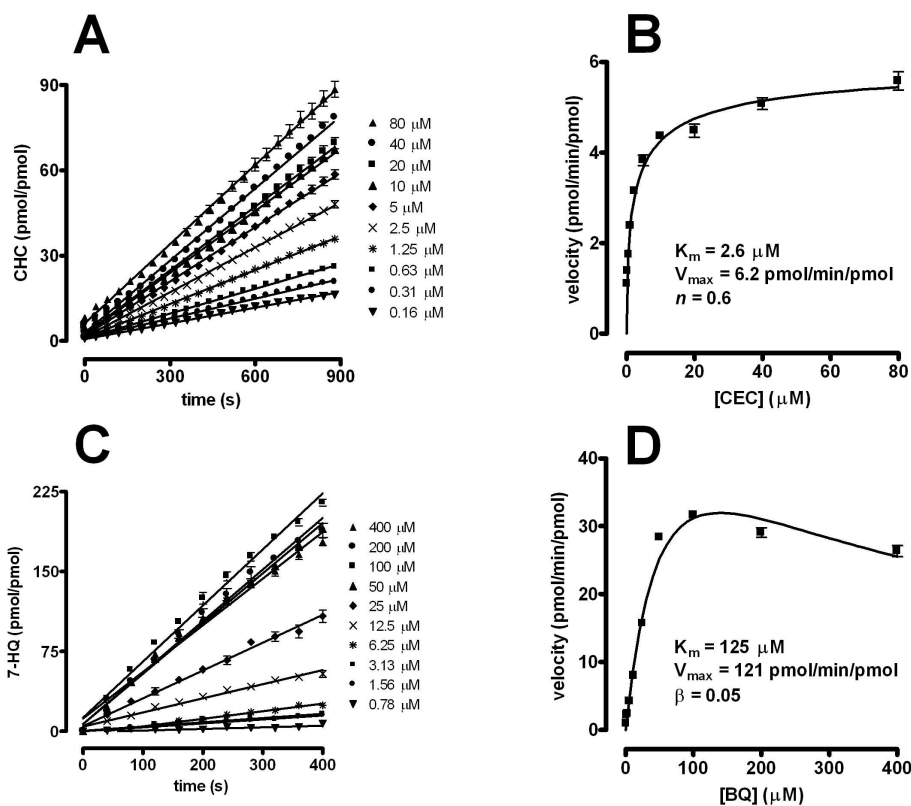


Figure 1.

Effect of substrate concentration on the biotransformation of fluorogenic substrates by human cytochromes P450 1A2 (A and B) and 3A4 (C and D). (A) The time course of formation of CHC from CEC by CYP1A2 was determined at ten concentrations of substrate by measuring the fluorescence intensity every 40 s over a 15 min period as described in Methods. Lines are linear least-squares regressions. (B) Concentration / velocity plot of the data in (A). (C) The time course of formation of 7-HQ from 7-BQ by CYP3A4 was determined at ten concentrations of substrate by measuring the fluorescence intensity every 40 s over a 7 min period as described in Methods. Lines are linear least-squares regressions. (D) Concentration / velocity plot of the data in (C). The line shows the fit of the data to equation (3). Each point represents the mean  $\pm$  SEM of triplicate observations in a single experiment.

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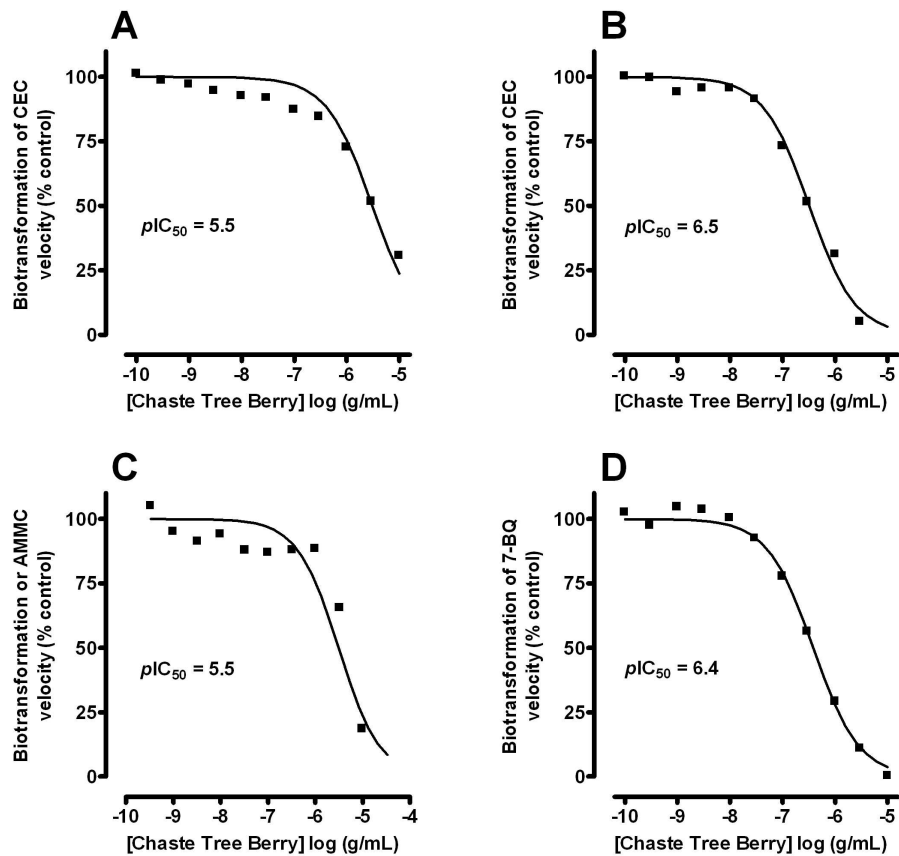


Figure 2.

The effects of increasing concentrations of chaste tree berry on the biotransformation of (A) CEC by CYP1A2, (B) CEC by CYP2C19, (C) AMMC by CYP2D6 and (D) 7-BQ by CYP3A4 as described in methods. The line of best fit was determined from the data by equation (5). Points are means + SEM of duplicates in a single experiment.

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