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Interactions of Papua New Guinea medicinal plant extracts with antiretroviral therapy

Erica C. Larson¹, Laura B. Hathaway¹, John G. Lamb¹, Chris D. Pond¹, Prem P. Rai², Teatulohi K. Matainaho^{2,3}, Pius Piskaut³, Louis R. Barrows^{1,2,3,*}, and Michael R. Franklin¹

Erica C. Larson: erica.c.larson@utah.edu; Laura B. Hathaway: laura.hathaway@utah.edu; John G. Lamb: greg.lamb@hci.utah.edu; Chris D. Pond: chris.pond@hsc.utah.edu; Prem P. Rai: raipp@yahoo.com; Teatulohi K. Matainaho: lmatainaho@yahoo.com; Pius Piskaut: piskautp@upng.ac.pg; Louis R. Barrows: lbarrows@pharm.utah.edu; Michael R. Franklin: michael.franklin@pharm.utah.edu

¹Department of Pharmacology and Toxicology, University of Utah, 30 S. 2000 E., Salt Lake City, Utah 84112, USA

²School of Medicine and Health Sciences, University of Papua New Guinea, PO Box 5623, Boroko, NCD, Papua New Guinea

³School of Natural and Physical Sciences, University of Papua New Guinea, PO Box 5623, Boroko, NCD, Papua New Guinea

Abstract

Ethnopharmacological relevance—A substantial proportion of the population in Papua New Guinea (PNG) lives with human immunodeficiency virus (HIV). Treatment requires lifelong use of antiretroviral therapy (ART). The majority of people in PNG use traditional medicines (TM) derived from plants for all types of health promotions. Consequently, there is a concern that herb-drug interactions may impact the efficacy of ART. Herb-drug, or drug-drug, interactions occur at the level of metabolism through two major mechanisms: enzyme induction or enzyme inhibition. In this study, extracts of commonly-used medicinal plants from PNG were screened for herb-drug interactions related to cytochrome P450s (CYPs).

Materials and Methods—Sixty nine methanol extracts of TM plants were screened for their ability to induce CYPs by human aryl hydrocarbon receptor- (hAhR-) and human pregnane X receptor- (hPXR-) dependent mechanisms, utilizing a commercially available cell-based luciferase reporter system. Inhibition of three major CYPs, CYP1A2, CYP3A4, and CYP2D6, was determined using human liver microsomes and enzyme-selective model substrates.

Results—Almost one third of the TM plant extracts induced the hAhR-dependent expression of CYP1A2, the hPXR-dependent expression of CYP3A4, or both. Almost two thirds inhibited

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*Address for correspondence: Prof. Dr. Louis R. Barrows, Department of Pharmacology and Toxicology, University of Utah, 30 S. 2000 E., Salt Lake City, Utah, 84112, USA, lbarrows@pharm.utah.edu.

Competing interests

The authors have no competing interests to disclose.

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CYP1A2, CYP3A4, or CYP2D6, or combinations thereof. Many plant extracts exhibited both induction and inhibition properties.

Conclusions—We demonstrated that the potent and selective ability of extracts from PNG medicinal plants to affect drug metabolizing enzymes through induction and/or inhibition is a common phenomenon. Use of traditional medicines concomitantly with ART could dramatically alter the concentrations of antiretroviral drugs in the body; and their efficacy. PNG healthcare providers should counsel HIV patients because of this consequence.

Keywords

Traditional medicine Asia & Oceania; herb-drug interactions; cytochrome P450s; Antiretroviral Therapy; Papua New Guinea

1. Introduction

Papua New Guinea (PNG) is in the midst of an HIV epidemic. According to the UNAIDS *2013 Report on the Global AIDS Epidemic*, an estimated 25,000 people in PNG are living with HIV (approximately 1% of the population) (UNAIDS, 2013). Standard treatment of HIV patients requires a lifelong regimen of antiretroviral therapy (ART). PNG is culturally diverse (Whitehead, 1994) and traditional medicines play a vital role in the well-being of the people (National Department of Health, 2007). Many people, especially in regions where access to Western medicine is limited, rely on medicinal plants to relieve an array of ailments (Rai, 2007). Medicinal herb-drug interactions have been studied in areas that rely heavily of traditional medicines around the world (Muller and Kanfer, 2011; Lau et al., 2013). However, little is known about herb-drug interactions that may arise from PNG medicinal plants in combination with ART.

Drug-drug interactions involving cytochrome P450s (CYPs) occur through two major mechanisms: enhanced enzyme expression, termed induction, and enzyme inhibition. With the exception CYP2D6, the CYPs responsible for drug metabolism in the liver are inducible. CYP induction and the resultant increased drug metabolism leads to a decrease in bioavailable drug and a decrease in drug efficacy. Inhibition of CYPs, on the other hand, decreases drug metabolism and results in an increase in bioavailability. Elevated levels of drugs may enhance their therapeutic benefits, but can result in toxicity.

Particularly worrisome is the possibility that traditional medicine usage may unknowingly render normal ART ineffective. We surveyed 69 methanol extracts of commonly-used medicinal plants from 7 PNG provinces for CYP induction and inhibition. Induction profiles were determined for CYP1A2 and CYP3A4, enzymes whose expression levels are elevated by two distinct pathways. The inhibition profiles were determined for CYP1A2, CYP3A4, and CYP2D6, which are estimated to be responsible for biotransformation of approximately two thirds of prescription drugs (Wrighton and Stevens, 1992). Almost one third of the medicinal plant extracts tested significantly induced CYP expression, while close to two thirds exhibited potent CYP inhibition.

2. Materials and Methods

2.1. Medicinal plant collection

As previously reported (Waruruai et al., 2011; Jorim et al., 2012), University of Papua New Guinea (UPNG) fourth year pharmacy students collected medicinal plants from their home communities. These were in the Eastern Highlands, Western Highlands, Southern Highlands, Enga, Western, Manus and Northern Bougainville (autonomous) provinces. These surveys served to fulfill requirements for a Bachelor of Pharmacy degree from UPNG. In accordance with the requirements, plant information, including local names and medicinal uses, was documented. Voucher samples were prepared for plant identification and the information was compiled into a formal report. Plants were identified by UPNG or National herbaria staff where vouchers are stored. The reported data are stored in the UPNG Traditional Medicines Database. A compilation of this information is presented in Appendix 1.

2.2. Medicinal plant extraction

Dried, plant samples (~10 g) were extracted in 100 mL of 100% methanol (MeOH) overnight. Extracts were then evaporated to dryness and dissolved in dimethyl sulfoxide (DMSO) to yield a final concentration of approximately 10 mg/mL. Fractionation of *Evodia hortensis* was accomplished by mixing MeOH extract with three times its dry weight of Diaion® HP20SS resin (Sorbent Technologies). The extract and resin mixture was evaporated to dryness, loaded into a column, and extracted using stepwise increases of isopropanol (IPA). The fractions were eluted as follows: 10% IPA/H₂O (FW), 25% IPA/H₂O (F1), 50% IPA/H₂O (F2), 75% IPA/H₂O (F3), and 100% MeOH (F4) (Bugni et al., 2008). These were evaporated to dryness and dissolved in DMSO to a final concentration of approximately 10 mg/mL.

2.3. CYP Induction Assays

Plant extracts were screened for the ability to induce CYP1A2 and CYP3A4. 1A2DRE (human XRE CYP1A2-luciferase promoter) and DPX2 (human PXRCYP3A4-luciferase promoter) hepatoma cell lines (Puracyp, Inc.) were grown in T75 flasks to near-confluency (~75%). The cells were trypsin-released and aliquoted into 96-well plates. Cells were allowed to adhere for 24 h. Plant extracts were added to the wells and incubated for another 24 h. Cell viability and luciferase activity were then determined using commercially-available kits (Promega). Viability was expressed relative to control wells exposed to DMSO (vehicle). Luciferase activity was normalized to cell number as determined from the viability assay; average result for quadruplicate determinations presented. Induction was compared to that seen for prototype inducers, 1 μ M β -naphthoflavone for 1A2DRE and 10 μ M rifampicin for DPX2. β -naphthoflavone produced ~10-fold induction and rifampicin produced ~8-fold induction. Criteria for “positive” plant samples, based on current Food and Drug Administration (FDA) guidelines and previously published methods, was set at ~40% of the response seen for the prototype inducers, *i.e.*, > 4.0-fold increase in luciferase activity for CYP1A2 and > 3.5-fold increase for CYP3A4 (FDA, 2012; Kuzbari et al., 2013).

2.4. CYP Inhibition Assays

Plant extracts were screened for inhibitory activity towards CYP1A2, CYP3A4, and CYP2D6 using enzyme-selective model substrates. Methoxyresorufin (MR) was used to assess CYP1A2 activity, 7-benzyloxyquinoline (7-BQ) was used to assess CYP3A4 activity and 7-methoxy-4-(aminomethyl)-coumarin (MAMC) was used to assess CYP2D6 activity. Plant extracts and human liver microsomes (Celsis) were added to 96-well plates (in duplicate, the average is reported). Substrate was added to the wells at half-saturating (K_m) or saturating (V_{max}) concentrations. NADPH (60 mM) was added to catalyze the enzymatic reactions and the formation of fluorescent product was measured using a Biotek – Synergy 2 Microplate Reader (Lamb et al., 2010). Extracts showing >50% inhibition at half-saturating and/or saturating substrate concentrations were deemed inhibitory. Whether the inhibition was suggestive of noncompetitive or competitive mechanism was noted. Noncompetitive inhibitors displayed similar inhibition at saturating and half-saturating substrate concentrations. Competitive inhibitors exhibited significantly greater inhibition at half-saturating substrate concentrations than at saturating substrate concentrations.

2.5. Characterization of *E. hortensis* extract sub-fractions

The methanol extract of *E. hortensis* inhibited all three CYPs tested. Therefore, the extract was fractionated as described above and fractions were re-tested for CYP inhibition. Fraction 4 inhibited all three CYPs. It was separated further using an Agilent Technologies 1200 series HPLC system. Prior to HPLC injection, Fraction 4 was evaporated to dryness and dissolved in DMSO. The sample was then injected onto a Phenomenex column (Luna 5 μ m C18 (2) 100Å; 250 $\times$ 10.00 mm). Sub-fractions were collected every 30 seconds at a flow rate of 2.5 mL/min and solvent gradient of 40% to 100% ACN/H₂O over 50 minutes. They were evaporated to dryness, dissolved in DMSO, and assayed for CYP inhibition.

3. Results

3.1. Inducers of CYP1A2 & CYP3A4

In our study, increases in luciferase activity reflected induction at the CYP1A2 and CYP3A4 promoters. This activity was compared to prototype inducers, β -naphthoflavone (CYP1A2) and rifampicin (CYP3A4). When luciferase activity was greater than 40% that of the prototype inducers, induction by the plant extract was considered significant (FDA, 2012). The results are summarized in Table 1. *Paspalum conjugatum* and *Sida rhombifolia* extracts induced exceptionally high levels of CYP1A2 expression (> 10-fold increase) but did not induce CYP3A4. Fourteen extracts induced CYP3A4 but not CYP1A2. These were: *Alstonia scholaris*, *Bidens pilosa* (em028 & cw004), *Coleus blumei*, *Cordyline terminalis*, *Curcuma longa*, *Manihot esculenta*, *Pometia pinnata*, *Psidium guajava* (sk002, nn011, in045, em009, & cw019), and *Wedelia biflora*. Five extracts induced both CYP1A2 and CYP3A4: *Calophyllum inophyllum*, *Codiaeum variegatum*, *Erythrina variegata*, *E. hortensis*, and *Sphaerostephanos unitus*. *C inophyllum* was a potent inducer of CYP1A2 and CYP3A4 (31.7-fold and 15.6 fold increase, respectively).

3.2. Inhibitors of CYP1A2, CYP3A4 & CYP2D6

Commercial preparations of human liver microsomes, enriched for the CYP of interest, were used to determine inhibitory activity. The ability of medicinal plant extracts to inhibit metabolism of CYP-specific substrates was determined at half-saturating (K_m) and saturating (V_{max}) substrate concentrations. Results are summarized in Table 2. Inhibitory activity that was similar at both substrate concentrations was interpreted as noncompetitive (Segel, 1968). Inhibitory activity that was greater at lower substrate concentrations was suggestive of competitive inhibition (Segel, 1968). Some plant extracts were selectively inhibitory towards one type of CYP: *Premna obtusifolia* and *S. rhombifolia* inhibited only CYP1A2. Inhibition by both extracts was suggestive of a competitive mechanism. Plant extracts that inhibited only CYP3A4, 10 total, all exhibited activity suggestive of a competitive mechanism. These were: *Angiopetris evecta*, *Carica papaya*, *Centella asiatica*, *Crinum asiaticum*, *C. terminalis* (jp1144), *Kalanchoe pinnata*, *Macaranga aleuritoides*, *M. esculenta*, *Passiflora foetida*, and *Premna serratofolia*. With respect to CYP2D6, *A. scholaris*, along with *Alstonia spectabilis*, *Citrus limon*, and *C. blumei*, showed selective inhibition suggestive of a noncompetitive mechanism. *B. pilosa* and *Solanum torvum*, showed selective inhibition suggestive of a competitive mechanism.

Some plant extracts were inhibitory towards two of the three CYPs tested. Six inhibited CYP1A2 and CYP3A4: *Acalypha* sp., *Ipomea pes-caprae*, *P. guajava* (nn011 & cw019), *S. unitus*, and *Syzygium malaccense*. *Acalypha* sp., *P. guajava* (nn011 & cw019), *S. unitus*, and *S. malaccense* showed inhibition suggestive of a noncompetitive mechanism for CYP1A2, whereas *I. pes-caprae* inhibited CYP1A2 in a manner indicative of a competitive mechanism. *I. pes-caprae* and *S. unitus* inhibited CYP3A4, suggesting a noncompetitive mechanism. Conversely, *Acalypha* sp., *P. guajava* (nn011 & cw019) and *S. malaccense* appeared to inhibit CYP3A4 by a competitive mechanism. *Cassia alata* (nn050) inhibited CYP1A2 in a manner suggestive of a competitive mechanism, while the same extract inhibited CYP2D6 under a noncompetitive mechanism. *C. terminalis* (cw030) and *C. variegatum* inhibited CYP3A4 and CYP2D6 by competitive mechanisms. *P. guajava* (in045) inhibited CYP3A4 competitively, however, the extract inhibited CYP2D6 in a manner suggestive of a noncompetitive mechanism.

Inhibition of CYP1A2, CYP3A4, and CYP2D6 could result in a broad impairment of drug metabolism. Ten plant extracts displayed this broad activity. *Casuarina equisetifolia*, *E. variegata*, *P. pinnata*, *P. guajava* (sk002) and *Terminalia catappa* inhibited CYP1A2, suggestive of a noncompetitive mechanism; whereas, *C. inophyllum*, *C. alata* (in014), *E. hortensis*, *P. conjugatum*, and *P. guajava* (em009) appeared to inhibit in a competitive fashion. *C. equisetifolia* inhibited CYP3A4, suggesting a noncompetitive mechanism, while the other nine plant extracts appeared to inhibit competitively. Only *T. catappa* inhibited CYP2D6 in a manner suggestive of a competitive mechanism. The other extracts inhibited CYP2D6 in a noncompetitive fashion, except for *C. alata* and *P. guajava* (sk002), which unexpectedly displayed less inhibition at the lower substrate concentration. The mechanism behind this observation is unknown.

3.3. Induction and Inhibition Overlap

Several extracts showed induction and inhibition towards various CYPs (Table 1). For instance, *S. rhombifolia* selectively induced and inhibited CYP1A2 and *M. esculenta* induced and inhibited only CYP3A4. *A. scholaris* and *C. blumei* only induced CYP3A4 expression and selectively inhibited CYP2D6. *P. guajava* and *C. terminalis* induced CYP3A4 and inhibited CYP3A4 and CYP2D6. *P. conjugatum* induced CYP1A2 and inhibited all three CYPs tested. *P. pinnata* induced CYP3A4 and inhibited all three CYPs.

C. inophyllum, *E. variegata*, and *E. hortensis* displayed broad inductive and inhibitory activity by inducing both CYP1A2 and CYP3A4 and inhibiting CYP1A2, CYP3A4, and CYP2D6. *S. unitus* induced and inhibited CYP1A2 and CYP3A4. *C. variegatum* induced both CYPs, but was inhibitory towards CYP2D6 and CYP3A4.

3.4. Inactive Plant Extracts

Several plant extracts did not exhibit either enzyme induction or enzyme inhibition. These are listed in Table 3.

3.5. Variable inhibition and induction results from the same plant

For some plants in the study, multiple samples had been collected from different locations. It was found that the induction and/or inhibition characteristics occasionally showed some degree of variation. Multiple reasons that extracts from different batches of plants might display inconsistent activities could include geographical region (Ozcan and Chalchat, 2005; Conforti et al., 2011), season of collection (dry or rainy) (Murakami et al., 2009), drying and extraction techniques (Komes et al., 2011), and the plant parts included in the extraction. A summary of the inductive and inhibitory properties of plants gathered from different regions with different plant parts listed is given in Table 4.

A. conyzoides, *E. indica* and *F. septica* were consistently non-active in both enzyme induction and enzyme inhibition assays, despite the different plant parts extracted. Among five leaf extracts of *P. guajava*, from different locations, all exhibited CYP3A4 induction and CYP3A4 inhibition; however, the inhibition of CYP1A2 and CYP2D6 varied (Table 4). Only one of three samples of *C. variegatum* showed significant enzyme-altering activity, as was the case for *C. papaya*. For the former, enzyme induction (CYP1A2 and CYP3A4) and inhibition (CYP3A4 and CYP2D6) was only seen in the sample from Manus, the two samples from North Bougainville were inactive. For *C. papaya*, one sample contained leaves only, one contained flowers, leaves and sap, and one contained whole plant. Only the sample composed of leaves inhibited CYP3A4.

3.6. Inhibitory characterization of *E. hortensis*

E. hortensis is a medicinal plant used for pain and inflammation in the Western Highlands Province of PNG. Screening results of *E. hortensis* revealed induction of CYP1A2 and CYP3A4 and inhibition of CYP1A2, CYP3A4, and CYP2D6 (Tables 1 & 2). Fractionation of the whole plant extract yielded high inhibitory activity towards all three CYPs in Fraction 4 (100% MeOH). Fraction 4 was further separated by HPLC. Interest was focused on the five major peaks that appeared in the 210 nm absorbance trace. HPLC fractions

corresponding to this region of the chromatogram was assessed for CYP inhibition. An expansion of the 210nm chromatogram of the five major peaks and inhibition data are aligned in Figure 1. Error bars indicate SEM.

Peak 1 (retention time (RT) - 18.78 min), Peak 2 (RT - 19.62 min), Peak 3 (RT - 20.20 min), and Peak 5 (RT - 22.95 min) showed preferential inhibition of CYP3A4. Peak 4 (RT - 21.38 min) preferentially inhibited CYP1A2. None of the fractions within this region inhibited CYP2D6.

4. Discussion

This investigation found that 21 out of 69 (~30%) of the PNG medicinal plant extracts tested induced CYP expression and 38 out of 69 (~55%) inhibited CYP-dependent metabolism. Many displayed both inhibition and induction, 18 out of 69 (~26%). Either outcome, alone or in combination, may impact the effective management of people on ART. Induction may have negative consequences by decreasing the overall concentrations of antiretroviral drugs in the body. Protease inhibitors are widely used in ART and are metabolized predominately by CYP3A4. Previous studies have shown that herbal remedies can reduce concentrations of protease inhibitors through CYP3A4 induction. St. John's Wort is an herbal supplement used to treat depression, it has been reported to significantly reduce plasma level of indinavir upon coadministration (Piscitelli et al., 2000). Of the medicinal plants tested, 19 of 69 significantly induced CYP3A4. Ritonavir has demonstrated drug-drug interactions due to CYP1A2 induction (Yeh et al., 2006). Seven of the 69 medicinal plant extracts also induced CYP1A2. The inductive herbal preparations reported here have the potential to attenuate the efficacy of antiretroviral and other drugs.

Inhibition can have either positive or negative consequences. Inhibition of CYPs in the presence of ART may lead to decreases in metabolism prolonging therapeutic effects. Ritonavir, a potent inhibitor of CYP3A4, is given in conjunction with other protease inhibitors to enhance their pharmacokinetic profiles and beneficial effects (Hsu et al., 1998; King et al., 2004; Josephson, 2010). Recently, a potent CYP3A4 inhibitor, cobicistat, has been specifically developed for the same purpose (Shah et al., 2013). Conversely, excessive CYP inhibition can have negative consequences due to toxicity associated with the elevated concentrations of antiretroviral or other drugs in the body (Bressler, 2006).

The medicinal plant extracts in our study displayed a range of inhibitory activities. Some extracts were selective towards individual CYPs, like *P. obtusifolia* towards CYP1A2. Other extracts exhibited broad inhibitory activity towards multiple CYPs, as in the case of *E. hortensis*. Characterization of *E. hortensis* revealed that multiple compounds within the extract contribute to its broad activity. The attribution of inhibition to a given metabolite in an extract is difficult because of the demonstrable presence of multiple inhibitory compounds. Inhibitory mechanisms of the extracts evaluated were suggestive of both competitive and noncompetitive inhibition.

These crude extracts provide a representative sampling of medicinal plants likely consumed by an HIV patient in PNG. The ability of plant extracts to induce and/or inhibit CYPs was determined *in vitro*. It is not known whether the active plant constituents exert their activity

in individuals consuming them. However, the data presented here suggest the “potential” of the identified medicinal plants to induce and/or inhibit ART metabolism in people living with HIV.

5. Conclusions

Medicinal plant usage in combination with ART is a likely scenario throughout much of PNG. As seen in this study, many PNG medicinal plants have the potential to induce and/or inhibit hepatic drug metabolizing enzymes, possibly resulting in unanticipated consequences on the management of HIV. For this reason, it is suggested that PNG healthcare providers counsel patients regarding concomitant medicinal plant usage, especially when a patient’s response to prescription therapy lies outside of that normally encountered. This knowledge will benefit PNG healthcare providers in managing the treatment regimens of their HIV patient population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Percent inhibition of CYP1A2, CYP3A4, and CYP2D6 following two-stage fractionation of an *E. hortensis* extract. Screening revealed inhibition of CYP1A2, CYP3A4, and CYP2D6. Fractionation of the methanol extract localized high inhibitory activity of all three CYPs in F4 (100% MeOH). F4 was separated by reversed-phase HPLC, collecting aliquots every 30 s over a 50 min run (H₂O:ACN 40% to 100%). Expansion of five major peaks from 210nm chromatogram and inhibition data are aligned; the complete chromatogram is inset. Error bars indicate S.E.M.

Table 1

CYP1A2 and CYP3A4 inducers and their inhibitory activity

Genus and Species	Voucher Number	Plant Part	PNG Province	CYP1A2 Induction (-fold)	CYP3A4 Induction (-fold)	Inhibits CYP1A2	Inhibits CYP3A4	Inhibits CYP2D6
<i>P. conjugatum</i>	cw070	spikelets	Western Highlands	11.4	--	✓	✓	✓
<i>S. rhombifolia</i>	nn010	leaves	Manus	15.5	--	✓		
<i>A. scholaris</i>	in012	leaves	North Bougainville	--	4.7			✓
<i>B. pilosa</i>	em028	leaves	Southern Highlands	--	5.7			
<i>B. pilosa</i>	cw004	leaves	Western Highlands	--	5.5			✓
<i>C. blumei</i>	sk043	whole plant	Eastern Highlands	--	4.0			✓
<i>C. terminalis</i>	cw030	leaves from shoots	Western Highlands	--	3.8		✓	✓
<i>C. longa</i>	jp1103	leaves	Enga	--	5.8			
<i>M. exculenta</i>	cw023	leaves	Western Highlands	--	3.7		✓	
<i>P. pinnata</i>	ab057	bark	Western	--	4.2	✓	✓	✓
<i>P. guajava</i>	sk002	leaves	Eastern Highlands	--	5.2	✓	✓	✓
<i>P. guajava</i>	nn011	leaves	Manus	--	4.6	✓	✓	
<i>P. guajava</i>	in045	leaves	North Bougainville	--	8.7		✓	✓
<i>P. guajava</i>	em009	leaves	Southern Highlands	--	5.8	✓	✓	✓
<i>P. guajava</i>	cw019	leaves	Western Highlands	--	3.5	✓	✓	
<i>W. biflora</i>	in021	leaves	North Bougainville	--	3.8			
<i>C. inophyllum</i>	nn021	leaves	Manus	31.7	15.6	✓	✓	✓
<i>C. variegatum</i>	nn016	leaves	Manus	5.8	14.2		✓	✓
<i>E. variegata</i>	nn056	bark	Manus	4.5	3.6	✓	✓	✓
<i>E. hortensis</i>	cw049	leaves & fruits	Western Highlands	13.7	7.1	✓	✓	✓
<i>S. unites</i>	in061	shoots	North Bougainville	14.9	5.0	✓	✓	

Medicinal plant induction of CYP1A2 and/or CYP3A4. (–) activity was < 4.0-fold (CYP1A2) & < 3.5-fold (CYP3A4); (✓) indicates inducers that also inhibited CYP metabolism.

Table 2

CYP1A2, CYP3A4, & CYP2D6 Inhibitors

Genus and Species	Voucher Number	Plant Part	PNG Province	CYP1A2 % Inhibition		CYP3A4 % Inhibition		CYP2D6 % Inhibition	
				MR (2.0 µM)	MR (0.4 µM)	7-BQ (500 µM)	7-BQ (100 µM)	MAMC (100 µM)	MAMC (40 µM)
<i>P. obtusifolia</i>	mn031	leaves	Manus	53	68	--	--	--	--
<i>S. rhombifolia</i>	mn010	leaves	Manus	54	75	--	--	--	--
<i>A. evecta</i>	cw055	leaves	Western Highlands	--	--	--	50	--	--
<i>C. papaya</i>	em016	leaves	Southern Highlands	--	--	54	78	--	--
<i>C. asiatica</i>	jp1165	whole plant	Enga	--	--	--	56	--	--
<i>C. asiaticum</i>	in059	leaves	North Bougainville	--	--	--	65	--	--
<i>C. terminalis</i>	jp1144	leaves	Enga	--	--	--	76	--	--
<i>K. pinnata</i>	mn019	leaves	Manus	--	--	--	55	--	--
<i>M. aleuritoides</i>	in002	leaves & shoots	North Bougainville	--	--	--	50	--	--
<i>M. esculenta</i>	cw023	leaves	Western Highlands	--	--	--	65	--	--
<i>P. foetida</i>	mn034	twigs	Manus	--	--	--	51	--	--
<i>P. serratifolia</i>	in005	leaves	North Bougainville	--	--	--	63	--	--
<i>A. scholaris</i>	in012	leaves	North Bougainville	--	--	--	--	--	53
<i>A. spectabilis</i>	ab005	bark	Western	--	--	--	--	72	81
<i>B. pilosa</i>	cw004	leaves	Western Highlands	--	--	--	--	--	51
<i>C. limon</i>	em010	fruits	Southern Highlands	--	--	--	--	53	61
<i>C. blumei</i>	sk043	whole plant	Eastern Highlands	--	--	--	--	--	57
<i>S. torvum</i>	in017	leaves	North Bougainville	--	--	--	--	--	52
<i>Acabypha sp.</i>	cw043	leaves from shoots	Western Highlands	85	71	--	71	--	--
<i>I. pes-caprae</i>	mn036	leaves	Manus	56	69	58	60	--	--
<i>P. guajava</i>	mn011	leaves	Manus	--	55	--	62	--	--
<i>P. guajava</i>	cw019	leaves	Western Highlands	58	66	--	66	--	--
<i>S. unguis</i>	in061	shoots	North Bougainville	60	65	53	--	--	--
<i>S. malaccense</i>	mn040	bark	Manus	56	--	--	60	--	--
<i>C. alata</i>	mn050	leaves	Manus	--	54	--	--	56	61
<i>C. variegatum</i>	mn016	leaves	Manus	--	--	67	97	50	60

Genus and Species	Voucher Number	Plant Part	PNG Province	CYP1A2 % Inhibition		CYP3A4 % Inhibition		CYP2D6 % Inhibition	
				MR (2.0 µM)	MR (0.4 µM)	7-BQ (500 µM)	7-BQ (100 µM)	MAMC (100 µM)	MAMC (40 µM)
<i>C. terminalis</i>	cw030	leaves from shoots	Western Highlands	--	--	--	88	50	61
<i>P. guajava</i>	in045	leaves	North Bougainville	--	--	--	50	51	51
<i>C. inophyllum</i>	nn021	leaves	Manus	74	86	--	79	52	60
<i>C. alata</i>	in014	leaves	North Bougainville	58	75	--	57	54	--
<i>C. equisetifolia</i>	nn026	bark	Manus	66	65	54	61	--	54
<i>E. variegata</i>	nn056	bark	Manus	74	74	57	85	66	64
<i>E. hortensis</i>	cw049	leaves & fruits	Western Highlands	52	69	93	100	--	51
<i>P. conjugatum</i>	cw070	spikelets	Western Highlands	77	87	60	88	57	53
<i>P. pinnata</i>	ab057	bark	Western	82	81	72	99	67	64
<i>P. guajava</i>	sk002	leaves	Eastern Highlands	52	57	53	85	59	--
<i>P. guajava</i>	em009	leaves	Southern Highlands	57	72	55	79	52	58
<i>T. catappa</i>	nn022	bark	Manus	96	92	64	80	67	81

Medicinal plant extract inhibition of CYP metabolism using CYP-specific substrate: methoxyresorufin (MR), 7-benzoxiquinolone (7-BQ), 7-methoxy-4-(aminomethyl)-coumarin (MAMC), (--) activity was < 50% inhibition.

Table 3

List of inactive plant extracts

Genus and Species	Voucher Number	Plant Part	PNG Province
<i>A. conyzoides</i>	sk013	leaves & flowers	Eastern Highlands
<i>A. conyzoides</i>	em021	leaves	Southern Highlands
<i>B. cernua</i>	cw085	leaves	Western Highlands
<i>C. papuana</i>	em036	bark	Southern Highlands
<i>Cyathea</i> sp.	jp1160	leaves	Enga
<i>E. indica</i>	nn012	leaves	Manus
<i>E. indica</i>	in060	whole plant	North Bougainville
<i>E. hirta</i>	nn020	whole plant	Manus
<i>F. copiosa</i>	em023	leaves	Southern Highlands
<i>F. septica</i>	in008	bark	Manus
<i>F. septica</i>	nn006	leaves & shoots	North Bougainville
<i>F. wasa</i>	cw032	leaves	Western Highlands
<i>H. rosa-sinensis</i>	em053	twigs	Southern Highlands
<i>H. tiliaceus</i>	in018	shoots	North Bougainville
<i>L. decumana</i>	jp1157	leaves	Enga
<i>M. peltata</i>	in041	leaves & shoots	North Bougainville
<i>M. micrantha</i>	in016	whole plant	North Bougainville
<i>M. citrifolia</i>	in007	leaves & fruits	North Bougainville
<i>Passiflora</i> sp.	jp1111	flowers & fruits	Enga
<i>R. klossii</i>	em055	whole plant	Southern Highlands

Induction activity was < 4.0-fold (CYP1A2) & < 3.5-fold (CYP3A4); inhibition activity was < 50% inhibition

Table 4

Activity of replicate plant extracts listed by plant part and collection site

Genus and Species	Voucher Number	Plant Part	PNG Province	CYP1A2 Induction (-fold)	CYP3A4 Induction (-fold)	CYP1A2 % Inhibition		CYP3A4 % Inhibition		CYP2D6 % Inhibition	
						MR (2.0 µM)	MR (0.4 µM)	7-BQ (500 µM)	7-BQ (100 µM)	MAMC (100 µM)	MAMC (40 µM)
<i>A. conyzoides</i>	sk013	leaves & flowers	Eastern Highlands	--	--	--	--	--	--	--	--
<i>A. conyzoides</i>	em021	leaves	Southern Highlands	--	--	--	--	--	--	--	--
<i>A. evecta</i>	em046	vines	Southern Highlands	--	--	--	--	--	--	--	--
<i>A. evecta</i>	cw055	leaves	Western Highlands	--	--	--	--	--	50	--	--
<i>B. pilosa</i>	em028	leaves	Southern Highlands	--	5.7	--	--	--	--	--	--
<i>B. pilosa</i>	cw004	leaves	Western Highlands	--	5.5	--	--	--	--	--	51
<i>C. papaya</i>	in032	whole plant	North Bougainville	--	--	--	--	--	--	--	--
<i>C. papaya</i>	em016	leaves	Southern Highlands	--	--	--	--	54	78	--	--
<i>C. papaya</i>	cw026	flowers, leaves & white sap	Western Highlands	--	--	--	--	--	--	--	--
<i>C. alata</i>	nn050	leaves	Manus	--	--	--	54	--	--	56	61
<i>C. alata</i>	in014	leaves	North Bougainville	--	--	58	75	--	57	54	--
<i>C. asiatica</i>	jp1165	whole plant	Enga	--	--	--	--	--	56	--	--
<i>C. asiatica</i>	nn014	leaves	Manus	--	--	--	--	--	--	--	--
<i>C. asiatica</i>	cw069	leaves	Western Highlands	--	--	--	--	--	--	--	--
<i>C. variegatum</i>	nn016	leaves	Manus	5.8	14.2	--	--	67	97	50	60
<i>C. variegatum</i>	in031	leaves	North Bougainville	--	--	--	--	--	--	--	--
<i>C. variegatum</i>	in006	leaves, stalk & sap	North Bougainville	--	--	--	--	--	--	--	--
<i>C. terminalis</i>	jp1144	leaves	Enga	--	--	--	--	--	76	--	--
<i>C. terminalis</i>	cw030	leaves from shoots	Western Highlands	--	3.8	--	--	--	88	50	61
<i>E. indica</i>	nn012	leaves	Manus	--	--	--	--	--	--	--	--
<i>E. indica</i>	in060	whole plant	North Bougainville	--	--	--	--	--	--	--	--
<i>F. septica</i>	in008	bark	Manus	--	--	--	--	--	--	--	--
<i>F. septica</i>	nn006	leaves & shoots	North Bougainville	--	--	--	--	--	--	--	--
<i>P. guajava</i>	sk002	leaves	Eastern Highlands	--	5.2	52	57	53	85	59	--
<i>P. guajava</i>	nn011	leaves	Manus	--	4.6	--	55	--	62	--	--

Genus and Species	Voucher Number	Plant Part	PNG Province	CYP1A2 Induction (- fold)	CYP3A4 Induction (- fold)	CYP1A2 % Inhibition		CYP3A4 % Inhibition		CYP2D6 % Inhibition	
						MR (2.0 µM)	MR (0.4 µM)	7-BQ (500 µM)	7-BQ (100 µM)	MAMC (100 µM)	MAMC (40 µM)
<i>P. guajava</i>	in045	leaves	North Bougainville	--	8.7	--	--	--	50	51	51
<i>P. guajava</i>	em009	leaves	Southern Highlands	--	5.8	57	72	55	79	52	58
<i>P. guajava</i>	cw019	leaves	Western Highlands	--	3.5	58	66	--	66	--	--
<i>S. torvum</i>	jp1126	whole plant	Enga	--	--	--	--	--	--	--	--
<i>S. torvum</i>	in017	leaves	North Bougainville	--	--	--	--	--	--	--	52

CYP Induction: (--) activity was < 4.0-fold (CYP1A2) & < 3.5-fold (CYP3A4); CYP Inhibition: (--) activity was < 50% inhibition; methoxyresorufin (MR), 7-benzoxiquinolone (7-BQ), 7-methoxy-4-(aminomethyl)-coumarin (MAMC).