Spring 5-9-2020

An Assessment of Herb-Drug Interaction Potential of Two Herbs: Momordica Charantia and Phyllanthus Amarus

Faridah Salau

AN ASSESSMENT OF HERB-DRUG INTERACTION POTENTIAL OF TWO HERBS: *MOMORDICA CHARANTIA AND PHYLLANTHUS AMARUS*

by
Faridah Salau

A thesis submitted to the faculty of the University of Mississippi in partial fulfillment of the requirements of the Sally McDonnell Barksdale Honors College

Oxford
May 2020

Approved by:

_____
Advisor: Dr. Shabana Khan

_____
Reader: Dr. Saumen Chakraborty

_____
Reader: Dr. Gregory Tschumper

ACKNOWLEDGEMENTS

I would like to thank my research advisor, Dr. Shabana Khan, for dedicating her time to helping me with this project. Without her support and input, this project would not have been possible. I would also like to thank Dr. Mona Haron for teaching me lab skills and for her help in many ways throughout the completion of this work. I am also grateful for my readers, Dr. Saumen Chakraborty and Dr. Gregory Tschumper, for taking time out their schedules to help me. Finally, I would like to thank my family for the love and support during the writing process. I could not have done this without them.

ABSTRACT

The use of natural supplements alongside prescribed medications is on the increase. Thus, it is important to analyze different properties of herbal supplements that can be implicated in eliciting herb-drug interactions. The herb *Momordica charnatia* is used throughout the world for its antidiabetic properties, and the herb *Phyllanthus amarus* is used in tropical and subtropical areas of the world for its hepatoprotective properties. Pregnane X Receptor (PXR) activation capabilities of these herbs was measured using a reporter gene assay. Additionally, Cytochrome P450 (CYP) isoform induction potential for CYP1A2, CYP3A4, CYP2B6, and CYP2C9 was determined through an enzymatic assay in hepatic cell line. *M. charantia* and *P. amarus* both increased PXR activity levels by a factor greater than two. Additionally, all four CYP isoforms were significantly induced by the herbs. These results indicate that when consumed concomitantly with conventional medicines both herbs may pose a risk of eliciting herb-drug interactions. Further studies in more advanced models are warranted to determine the clinical relevance of these *in vitro* findings.

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

LIST OF ABBREVIATIONS

ART	Antiretroviral Treatment
ATCC	American Type Culture Collection
CAM	Complementary and Alternative Medicine
CAR	Constitutive Adrostane Receptor
CYP	Cytochrome P450
FBS	Fetal Bovine Serum
HEPG2	Human Hepatocellular Carcinoma Cells
NNRTI	Non-nucleoside Reverse Transcriptase Inhibitor
PXR	Pregnane X Receptor

1. INTRODUCTION

In 2012, a national survey was conducted in which it was revealed that greater than 30% of US adults used some form of complementary and alternative medicine (CAM)[7]. CAM includes but is not limited to acupuncture, meditation, Ayurveda, herbal medicine, and dietary supplements[37]. Natural products, classified as a dietary supplement that was not a vitamin or mineral, were found to be the most utilized CAM at 17.7%[7]. A separate study found that patients with chronic diseases as well as those who use over the counter drugs had an increased likelihood of using herbal supplements[28].

Presently, the FDA's regulation of herbal supplements is not as strict as the regulation of drugs. Herbal supplements are not required by law to meet the FDA's safety requirements before they are marketed to the general public[6]. Therefore, the FDA is only legally able to act against companies once a product has reached the public and has been shown to induce illness or cause harm. Because of the prevalence of supplement usage alongside allopathic methods, it is critical to analyze the interactions between drugs and herbal supplements. One well-known example of an herb that demonstrates several herb-drug interactions is St. John's wort (*Hypericum perforatum*). This herb is commonly used in dietary supplements for treating anxiety and depression[31]. However, its use is associated with a reduction in the effectiveness of

the anti-coagulant drug warfarin as well as oral contraceptive pills through the induction of cytochrome P450 (CYP) enzymes[3,26]. By evaluating the effect of different supplements on the drug metabolizing enzymes and the pharmacokinetics of conventional drugs and disseminating this information to the general public, both consumers and practitioners can be more aware of the potential threats seemingly harmless herbs may pose to one's dosing regimen.

Two herbal supplements namely *Phyllanthus amarus* (*P. amarus*) and *Momordica charantia* (*M. charantia*) were selected for this study and evaluating their influence on the activity of major drug metabolizing enzyme CYP450 isoforms was the primary focus of this study. Pregnane X receptor (PXR) is a nuclear receptor that acts as a transcription factor and regulates the expression of genes responsible for drug metabolizing enzymes and transporters. Activation of PXR has been found to be responsible for clinically relevant pharmacological drug interactions due to an increase in the activity of major drug metabolizing enzymes such as CYP3A4 and CYP2C9 that results into enhanced clearance of drugs making them less effective[15]. Previous studies have shown that both herbal supplements, *P. amarus* and *M. charantia,* have a significant effect on the induction of PXR activity levels when tested at 60 µg/mL[12]. It is important to determine whether these results are indicative of either herb's capability to induce the activity of drug metabolizing enzymes known to mediate herb-drug interactions. This will help in predicting the potential of these herbs to pose a risk of herb-drug interaction if they are consumed along with the prescription drugs for a long time (chronic exposure) or consumed in high quantities (acute exposure).

P. amarus, commonly known as 'Carry Me Seed', is popularly used as traditional medicine in tropical and subtropical parts of the world such as the Amazon, India, and Malaysia. It is used in ayurvedic medicine to confront an array of conditions associated with the stomach, kidney, spleen, and liver[27]. Additionally, *P. amarus* has been known to display hepatoprotective properties. Enogieru et al. (2015) found that in adult wistar rats the presence of *P. amarus* and the amount of liver damage suffered due to acetaminophen intake were negatively correlated.

M. charantia also known as bitter melon, is consumed as a culinary item for its multitude of nutritious properties such as a high concentration of carotenoids[19]. Additionally, it is used alongside or instead of western medication for its perceived antidiabetic properties in parts of East Asia, East Africa, India, and South America[18].

Drugs and herbal supplements can be referred to as xenobiotics, defined by Dictionary.com as "a chemical or substance that is foreign to an organism or biological system." The majority of xenobiotic metabolism occurs within the liver. The protein pregnane x receptor (PXR) has been highlighted alongside constitutive androstane receptor (CAR) as a xenobiotic sensing receptor present in the liver[20]. Because of the large, hydrophobic, and flexible ligand-binding domain, PXR is capable of being activated by a multitude of compounds leading to this being a major pathway of xenobiotic metabolism[38]. It serves as a transcription factor for a large number of CYP enzymes including CYP2C9, CYP3A4, CYP2B6[33]. PXR is activated following the binding of a ligand. Once the ligand activates PXR, the expression of CYP enzymes is induced[21].

According to Horn and Hansten (2007), CYP enzymes are aggregated mainly in the liver and serve to synthesize molecules such as cholesterol and steroid hormones. Additionally, it has been shown that these enzymes serve to metabolize medications as well as autogenous toxins, and they are thought to be the leading mechanism through which pharmacokinetic interactions occur[16]. These enzymes belong to the superfamily of monooxygenases and are of utmost importance during phase I of xenobiotic metabolism[13]. During this stage, the enzymes' heme prosthetic group oxidizes hydrophobic compounds preparing the molecule for excretion[32].

CYP3A4, which aggregates in the small intestine in addition to the liver, is known as the CYP enzyme that metabolizes over 50% of drugs on the market[34]. If a product tested were to inhibit this cytochrome, then it would increase plasma concentrations of drugs normally metabolized by said cytochrome[17]. On the other hand, if the product tested were to induce the cytochrome, then plasma concentrations of the drug would decrease. This would cause the drug to become less effective, thus requiring higher dosages to be prescribed. These same processes could occur with the inhibition or induction of other key isoforms involved in drug metabolism such as CYP2B6, CYP2C9, and CYP1A2.

Rifampicin, a drug used in the treatment of tuberculosis, is a well-known example of a CYP enzyme inducer[4]. Through the activation of PXR, it has been found to induce a variety of cytochromes including CYP2B6, CYP3A4, and CYP2C9. For these reasons, it was used as a positive control during this study. According to the World Health Organization, people with HIV are 16-27 times more likely to develop tuberculosis than those without HIV. With Rifampicin being a first-line drug in the treatment of

tuberculosis, many studies have been performed to determine its drug-drug interactions with antiretroviral treatment (ART)[35].

An ART commonly used in the treatment of HIV is nevirapine, a non-nucleoside reverse transcriptase inhibitor (NNRTI)[22]. This drug is mainly metabolized through CYP3A4 with a small amount of metabolism by CYP2B6. Rifampicin induces CYP3A4 to a greater extent than CYP2B6, and it has been shown that when used concomitantly with rifampicin, the plasma concentration levels of antiretroviral drug nevirapine undergo a significant reduction[22]. In one study, 21% to 38% of HIV/tuberculosis co-infected patients being treated with both rifampicin and nevirapine showed subtherapeutic nevirapine plasma concentration levels[29]. Additionally, rifampicin has been shown to increase clearance times of the blood thinner warfarin[11]. Three times higher dosage of the immunosuppressive drug cyclosporine is required when used concomitantly with rifampicin due to the subtherapeutic levels of regular dosage under these conditions.

There are over fifty CYP enzyme isoforms, but most xenobiotics are metabolized through CYP3A4, CYP2D6, CYP2C9, CYP1A2, CYP2C8, CYP2C19, CYP2B6, and CYP2E1[9]. This study was carried out to determine the effects of *P. amarus* and *M. charantia* on four major enzymes namely : CYP3A4, CYP1A2, CYP2B6, and CYP2C9 which are responsible for the metabolism of a number of commonly consumed prescription drugs such as antiretroviral, anticoagulant, contraceptive, antidiabetic, and cardiovascular drugs.

2. MATERIALS AND METHODS

2.1 Materials

1. Cell Culture Medium
    a. This medium contained one packet of DMEM/F12 and 1% Pen-Strep purchased from Gibco as well as 2.4 g of sodium bicarbonate from Fisher. There was an addition of 10% fetal bovine serum (FBS) which was purchased from Atlanta Biologicals.
2. No Serum Medium
    a. It was prepared in the same manner as the culture medium with the exception of the addition of FBS.
3. Transfection Medium
    a. It was prepared in the same manner as the culture medium but contained 2.5% FBS.
4. Human hepatocellular carcinoma cells (HepG2)
    a. Purchased from the American Type Culture Collection (ATCC). As mentioned previously, the majority of xenobiotic metabolism occurs in the liver, thus it was most appropriate to use a liver cell line.
5. 0.25% Trypsin
    a. 2.5 g Trypsin purchased from Gibco was combined with 0.3 g disodium salt EDTA and 1.0 g glucose both purchased from Sigma-Aldrich. 8.0 g of

b. NaCl, 0.4 g of KCl, and 0.58 g sodium bicarbonate (all purchased from Fisher) were also added into the mixture.

6. Matrigel
    a. Purchased from Corning
7. *M. charantia* extract (methanolic extract)
8. *P. amarus* extract (methanolic extract)
9. 10 mM Rifampicin (Sigma -Aldrich)
10. DMSO (Fisher)
11. Promega P450-Glo Assay kits were used to detect the induction of CYP1A2, CYP3A4, CYP2B6, and CYP2C9 enzymatic activities.

2.2 Maintenance of Cells

HepG2 cells were retrieved from liquid nitrogen storage and thawed in a 37℃ water bath for about 1 minute. The outside of the vial containing the cells was disinfected using 70% ethanol, and then cells were transferred to a centrifuge tube. 9 mL of prewarmed culture medium was added to the 1 mL of cells in the centrifuge tube. Cells were centrifuged at 1000 rpm for 5 minutes. Once finished, the supernatant was discarded, and the cells were resuspended in 10 mL of culture medium and transferred to a culture dish pre-coated with Matrigel. The dish was incubated in a cell culture incubator at 37℃ in an atmosphere of 5% CO_2 and 95% humidity. Twenty four hours later, the culture medium was replaced with fresh medium and cells were incubated further. Once the cells were confluent (about 4 to 5 days later) cells were trypsinized, resuspended in culture medium and plated in a 50 mL culture dish containing Matrigel. They were incubated at 37℃ with 5% CO_2. Cells were subcultured twice a week once they reached

around 70% confluency in order to maintain integrity. Confluency was estimated by observing cells under a microscope. Subculturing consisted of five steps. The old culture medium was first removed from the culture dish. Cells were then dislodged from the Matrigel using 0.25% trypsin. Once the trypsin was aspirated from the culture dish, cells were incubated in 37℃ until completely detached from the culture dish. While the cells were incubating, a new culture dish coated with Matrigel was filled with fresh culture medium. Finally, the cells were resuspended in culture medium, aliquoted, and added into a new culture dish containing Matrigel and fresh culture medium. Cells were incubated either for three to four days before the next subculture or until needed for use.

2.3 Preparation of Plasmid DNA (PXR and PCR-5)

LB medium was made at a pH of 7.5 using 10 g of tryptone, 5 g of yeast, and 5 g of NaCl dissolved in 950 ml of Nanopure water. Medium was autoclaved at 121 ℃ for 15 minutes and stored at 4℃. 10 mg/ml Ampicillin was added to LB medium to make LBA medium. LBA agar plates were made by adding 3.75 g of Bactoagar to 250 ml of LB medium. This solution was autoclaved at 121 ℃ for 15 minutes, cooled down to 45℃, and then 2.5 mL of 5 mg/ml ampicillin were added. Agar was poured into petri dishes and once solidified stored at 4℃. Ca^{2+} DH5-∂ cells were thawed and mixed. 100 ul of cell was placed in a 12 x 75 mm chilled snap cap tube. 10 ng of plasmid DNA was added to the tube, and cells with plasmid were incubated on ice for 30 minutes. Cells were then heat-shocked for 1 minute at 37℃ and placed on ice for 2 minutes. 900 ul of LB medium was added to the tube which was then shaken for four hours at 225 rpm. Transformed cells were then diluted and 100 ul of each dilution was spread on LBA plates which were incubated overnight at 37℃. Using a sterile inoculum loop, a colony was transferred

from the agar plate to a snap-cap tube that contained 4 ml LBA medium. This was done at least three times using different colonies. Overnight, the tubes were shaken at 37℃ at 165 rpm. The tube with the greatest turbidity had l ml transferred to 3 sterile 1L flasks that contained 400 ml LBA medium. Flasks were shaken overnight at 180 rpm at 37℃. Each flask's content was then centrifuged at 37 ℃ at 3000 rpm for 30 minutes in a 500 ml plastic bottle. The pellet was retained.

Isolation of plasmid DNA from the pellet of transformed E. Coli was carried out using a Qiagen® Plasmid Purification Kit according to the instructions provided with the kit (www.qiagen.com/handbook" www.qiagen.com/handbook). Plasmid DNA was stored at 4^{o}C and used for transfecting HepG2 cells.

2.4 Plant Extracts Preparation

The dried powders of leaves of *M. charantia* and the whole plant (root stem and leaves) of *P. amarus* from the National Center for Natural Products Research repository were extracted in methanol four times over a 24-hour time period in room temperature. Methanolic solutions were evaporated to complete dryness using a speed vacuum system. This yielded dried extracts. Extracts were then transferred to clean containers and stored in tightly closed glass vials. Solutions were prepared in DMSO to a concentration of 20 mg/mL for the bioassays described below for PXR and CYPs activities.

2.5 Transfection of Cells

HepG2 cells were subcultured into a new plate the day before beginning the assay. Cells were subcultured in a manner that ensured confluency the next day. On the first day of the assay, cells were transfected with human PXR (hPXR) and the luciferase reporter plasmid PCR5[12,23]. First, the cells were trypsinized and placed in a 15 mL

centrifuge tube with 10 mL culture medium. 10 µL of the solution was placed in a cell counter slide. The slide was placed in an automated cell-counter in order to determine the cell concentration/mL. **Equation 1** was then used to determine the quantity of medium containing cells that would be needed for even distribution of about 50,000 cells/well in a 96-well plate. The calculated volume was then transferred to a new centrifuge tube. This solution was centrifuged for 5 minutes at 1000 rpm. The medium was then removed and replaced with 7 mL of DMEM/F12 medium (NS medium). Cells were resuspended in the medium, and the solution was centrifuged again for 5 minutes at 1000 rpm. The supernatant was removed, and the pellet was resuspended in 450 µL of transfection medium. 25 µg of hPXR and 25 µg of PCR5 were added to this solution.

After mixing, the solution was kept under the hood at room temperature for five minutes. The mixture was then transferred to an electroporation cuvette and transfected at 180V for 70 msec using one pulse on an electroporator. The cuvette was then left untouched for 8 minutes under the hood. A transfer pipette was used to remove the foam from the cuvette, and the cells were mixed with the predetermined volume of culture medium needed for a full 96-well plate. A volume of 190 µL of the cell suspension was pipetted into each well of the sterile, white-bottomed polystyrene 96-well plate. In a sterile, clear polystyrene 96-well plate 200 µL of cell suspension was placed in 3 wells to watch for contamination during the rest of the week. Each plate was incubated at 37°C for 24 hours.

2.7 Sample Addition

A. PXR induction assay

During the second day of the assay, the positive control (Rifampicin), negative control (DMSO), *Phyllanthus amarus* (PA), and *Momordica charantia* (MC) extracts were added into the wells at varying concentrations based on a predetermined template. In the wells of a sterile, clear polystyrene plate, samples of PA and MC were serially diluted (3 fold) with no serum media. The five concentrations were 60, 20, 6.6, and 2.2, and 0.6 µg/mL. A 6% DMSO + NS medium solution was made and used for the dilution of the 20, 6.6, and 2.2, and 0.6 µg/mL samples. Each concentration was made in a different well. In the same plate, 30 µM of Rifampicin was made. The 96-well white-bottomed polystyrene plate containing the transfected cells was retrieved from the incubator, and samples were added directly to the medium of their predetermined well. Each plate was again incubated for 24 hours at 37°C.

B. CYP induction assay

During the second and third days of the assay, the positive control (Rifampicin), negative control (DMSO), *Phyllanthus amarus* (PA), and *Momordica charantia* (MC) extracts were added into the wells at varying concentrations based on a predetermined template. In a sterile, clear polystyrene plate, the four concentrations of PA and MC samples were generated. The three concentrations were 30, 10, and 3.3 µg/mL. A 6% DMSO + NS medium solution was made and used for the dilution of the 10, 3.3, and 1.1 µg/mL samples. Each concentration was made in a different well. In the same plate, 30 µM of Rifampicin was made. Before adding the herbs and the controls, the medium was removed from each well without disrupting the cells and was replaced with 190 µL of serum medium. 10 µL of the samples, Rifampicin, and 6% DMSO were added to their

preset well. The clear control plate was checked for any signs of contamination. Each plate was incubated at 37°C for 24 hours.

2.8 PXR induction assay data procurement

After transfection of cells and one day of sample addition, results were procured through the measurement of luminescence. Once the 24-hour incubation period was over, the medium was aspirated from each well and replaced with 40 µL of luciferase mix to each well. The plate was immediately placed in a Spectramax M5 plate reader for luminescence reading. Fold induction in the luciferase activity of sample treated cells was then calculated in comparison to DMSO-treated cells (vehicle control).

2.8 CYP Induction assay data procurement

After transfection of cells and two days of sample addition, results were procured through the measurement of luminescence. Preparation of the wells was dependent upon the enzyme that was being tested. For CYP3A4 and CYP2C9, the medium was removed from the well, and the well was washed one time with 100 µL of PBS. In the CYP3A4 wells, 50 µL of 2.5% luciferin PFBE + culture medium was added to each well. CYP2C9 wells had 50 µL of 2.0% luciferin H + culture medium added to them. The plate was then incubated for 3 hours at 37°C. 40 µL of in house luciferase mix was added to each well after the incubation period, and the plate was read using a Spectramax M5 plate reader. Fold increase in luciferase activity was then calculated in sample treated cells in comparison to DMSO-treated cells (vehicle control).

For CYP2B6 and CYP1A2 enzymes, a 3 mM salicylamide solution in PBS was prepared. Once the salicylamide solution was prepared, the medium was removed from the wells. Wells were then washed two times with 50 µL of PBS. 50 µL 0.1% luciferin

2B6 + salicylamide solution was added into each of the CYP2B6 wells. In the CYP1A2 wells, 50 µL of 0.1% luciferin 1A2 +salicylamide solution was added. The cells being tested for CYP2B6 activity were incubated for 2 hours whereas those testing for the CYP1A2 activity were incubated for 1 hour. Once the incubation period for both enzymes was finished, 0.1% d-cysteine + luciferase mixture was made. 50 µL of this mixture was added into each well, and the plate was read using a Spectramax M5 plate reader. Fold increase in luciferase activity was then calculated in sample treated cells in comparison to DMSO-treated cells (vehicle control).

2.9 Statistical Methods

GraphPad Prism 8 was used. One-way ANOVA was utilized in the analyzation of data. This was followed by Dunnett's multiple comparison tests. Results were considered statistically significant if $p < 0.05$.

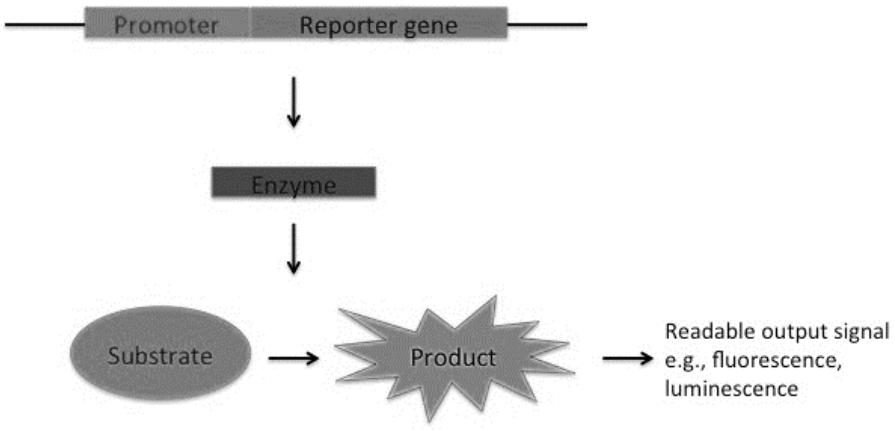


Figure 1: Diagrammatic representation of a Reporter Gene Assay (Karen Cohrt, 2018)

Equation 1: $$\textbf{volume of cells needed (mL)} = \frac{\frac{2.5\times10^5}{\textbf{mL}}(\textbf{total volume of media (mL) needed per white-bottomed plate})}{\frac{\textbf{Cell concentraion}}{\textbf{mL}}}$$

3. RESULTS AND DISCUSSION

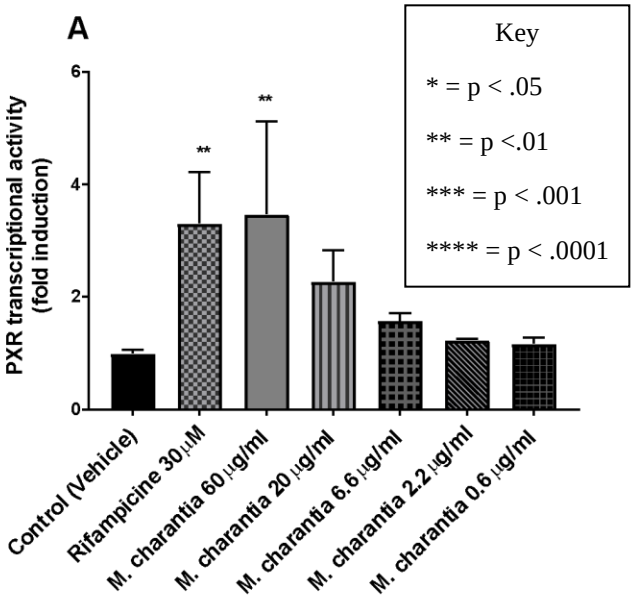


Figure 2: PXR activation by *M. charantia* methanolic extract. Values were determined using a luciferase gene assay in HepG2 cells. Rifampicin was the positive control. Data represents the mean ± SD.

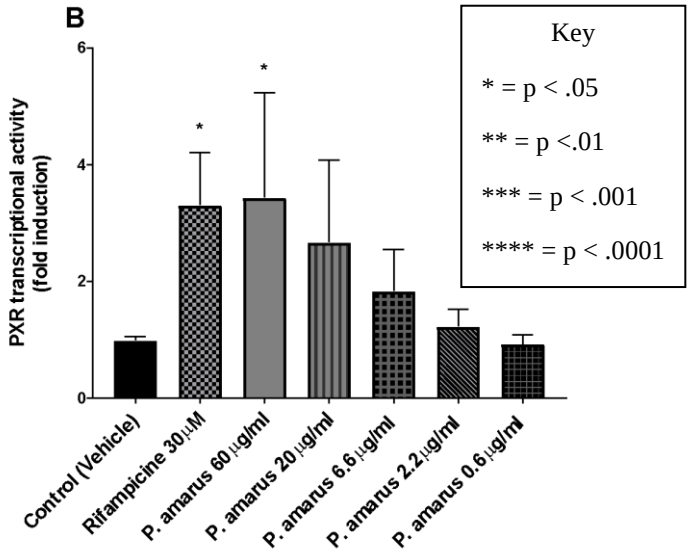


Figure 3: PXR activation by *P. amarus* methanolic extract. Values were determined using a luciferase gene assay in HepG2 cells. Rifampicin was the positive control. Data represents the mean ± SD.

Table 1: Comparison of PXR activation between *M. charantia* and *P. amarus*

Test concentration	*M. charnatia*	*P. amarus*
60 µg/mL	3.48 ± 1.65	3.44 ± 1.80
20 µg/mL	2.28 ± 0.56	2.67 ± 1.41
6.6 µg/mL	1.58 ± 0.13	1.84 ± 0.71
3.3 µg/mL	1.24 ± 0.02	1.23 ± 0.30
0.6 µg/mL	1.17 ± 0.11	0.93 ± 0.12

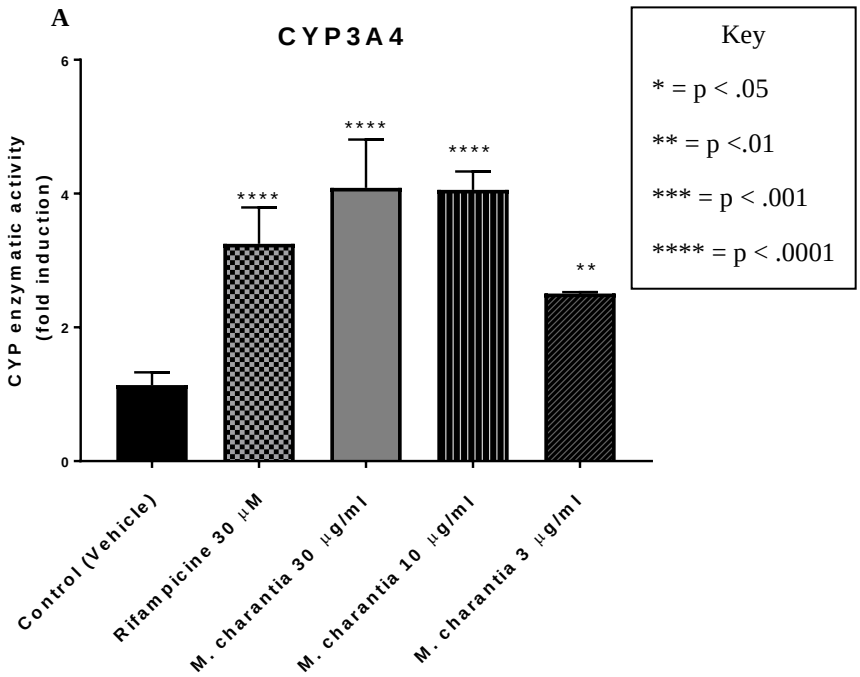


Figure 4: Increase in CYP3A4 enzyme activity by *M. charantia* methanolic extract in HepG2 cells. Rifampicin was the positive control. Data represents the mean ± SD.

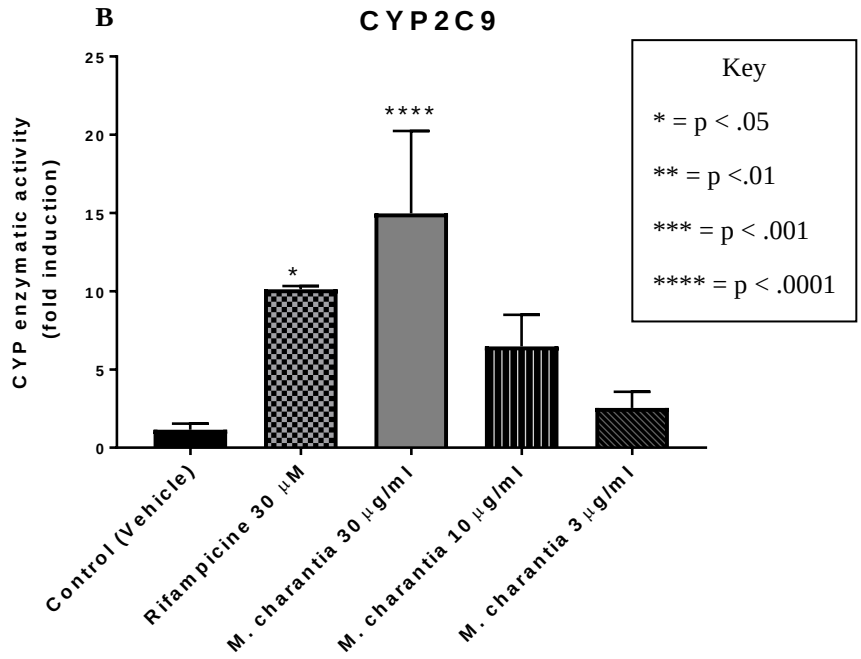


Figure 5: Increase in CYP2C9 activity by *M. charantia* methanolic extract. Rifampicin was the positive control. Data represents the mean ± SD.

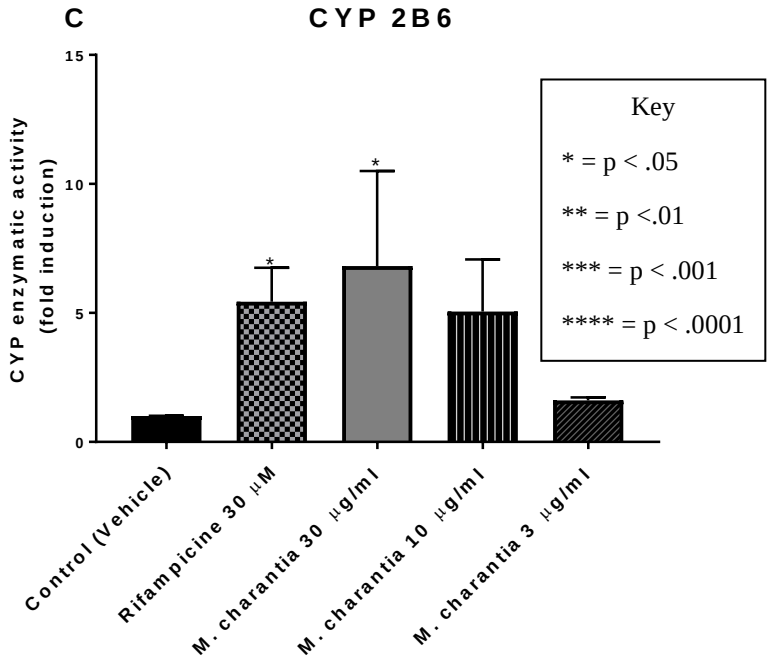


Figure 6: Increase in CYP2B6 activity by *M. charantia* methanolic extract in HepG2 cells. Rifampicin was the positive control. Data represents the mean ± SD.

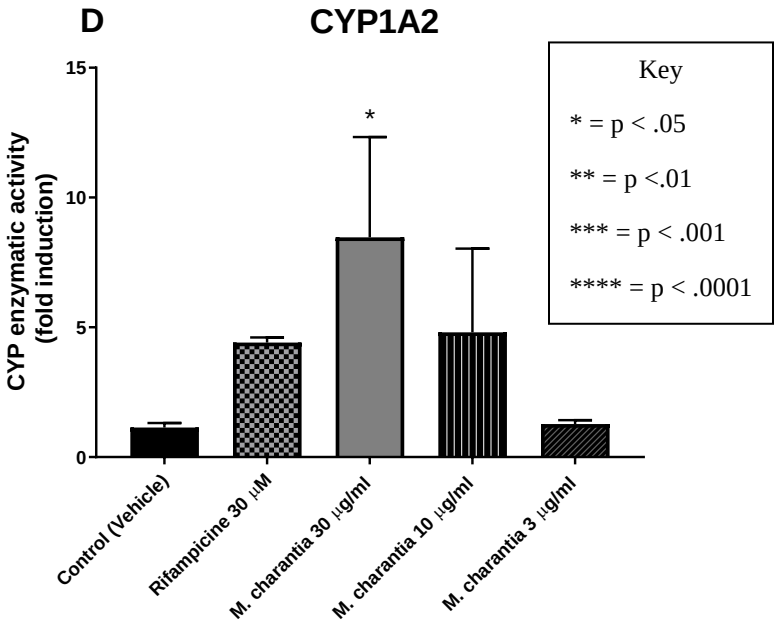


Figure 7: Increase in CYP1A2 activity by *M. charantia* methanolic extract in HepG2 cells. Rifampicin was the positive control for enzyme expression. Data represents the mean ± SD.

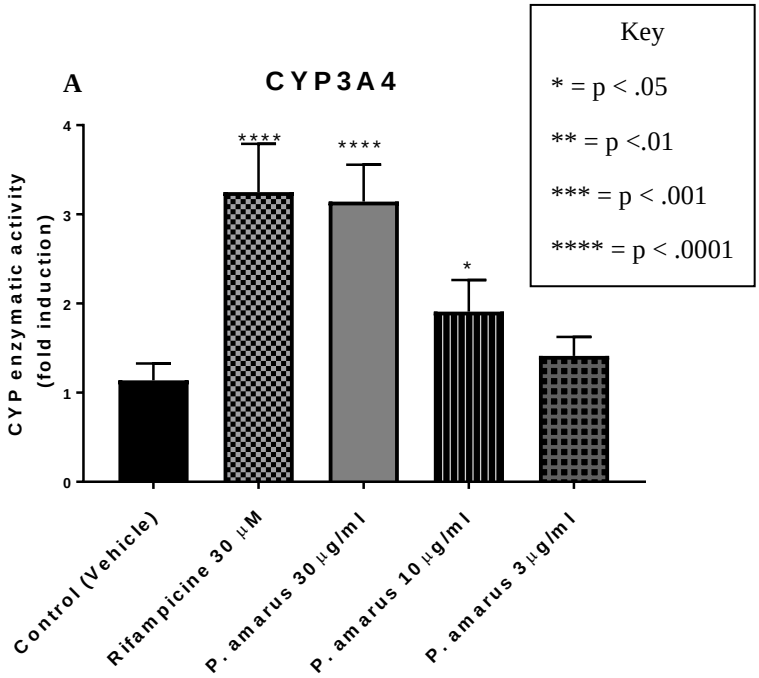


Figure 8: Increase in CYP3A4 activity by *P. amarus* methanolic extract in HepG2 cells. Rifampicin was the positive control. Data represents the mean ± SD.

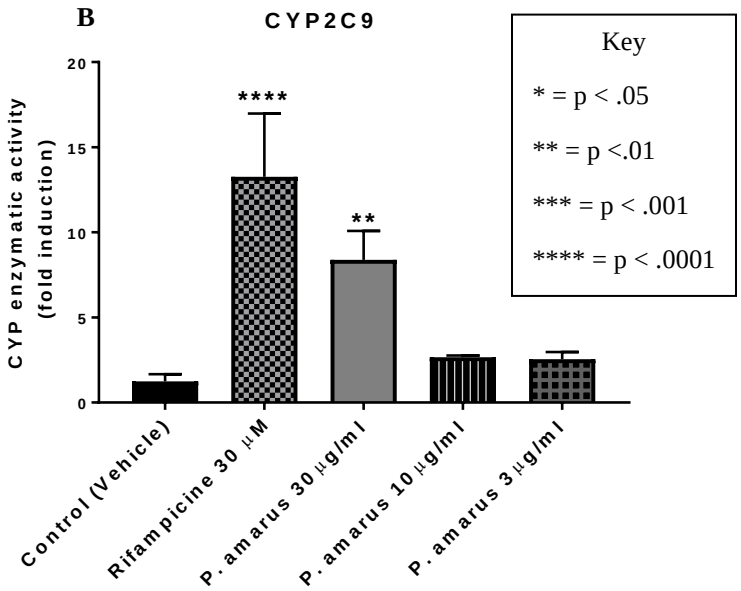


Figure 9: Increase in CYP2C9 activity by *P. amarus* methanolic extract in HepG2 cells. Rifampicin was the positive control. Data represents the mean ± SD.

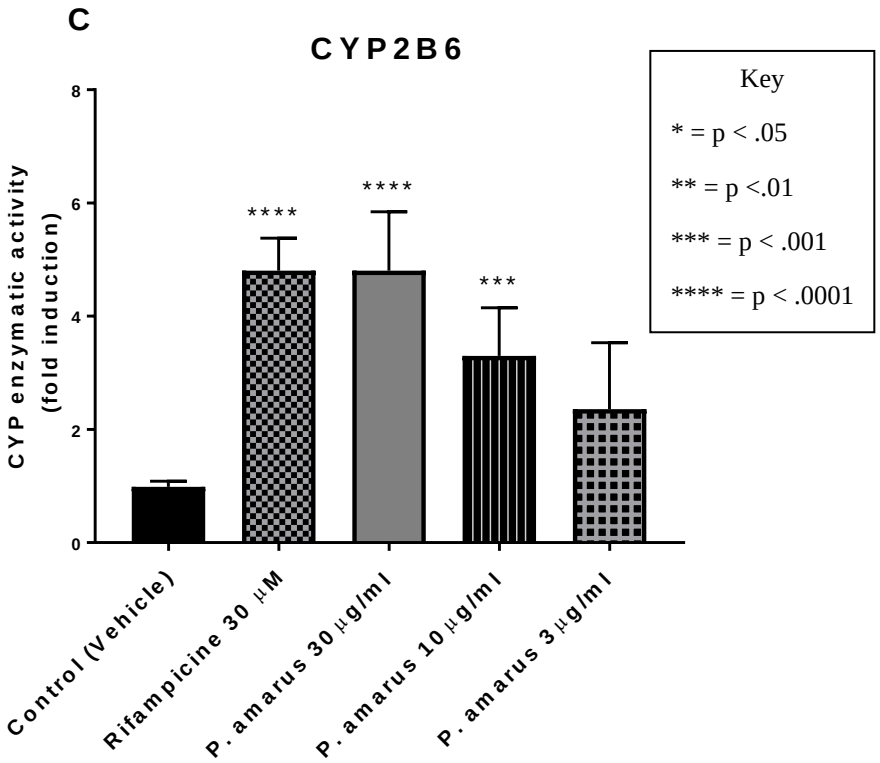


Figure 10: Increase in CYP2B6 activity by *P. amarus* methanolic extract in HepG2 cells. Rifampicin was the positive control. Data represents the mean ± SD.

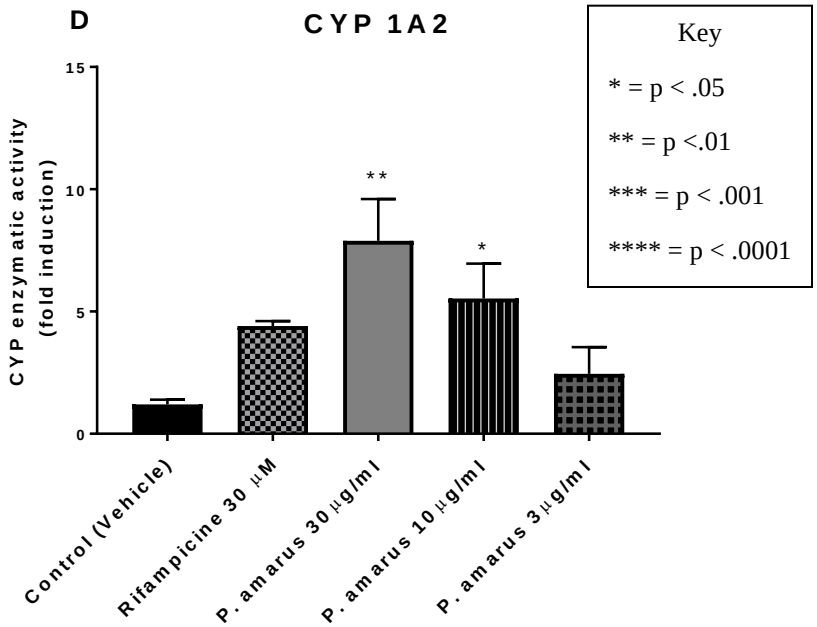


Figure 10: Increase in CYP1A2 activity by *P. amarus* methanolic extract in HepG2 cells. Rifampicin was the positive control. Data represents the mean ± SD.

Figure 11: *M. charantia*

(Sayat Arslanlioglu, 2008)

***Figure 12**: P. amarus*

(Jim Conrad, 2019)

Table 2: CYP3A4 fold induction comparison between M. charantia and P. amarus

Test concentration	M. charantia	P. amarus
30 µg/ml	4.09 ± 0.72	3.14 ± 0.41
10 µg/ml	4.06 ± 0.28	1.91 ± 0.35
3 µg/ml	2.51 ± 0.02	1.41 ± 0.21

Table 3: CYP2C9 fold induction comparison between M. charantia and P. amarus

Test concentration	M. charantia	P. amarus
30 µg/ml	14.97 ± 5.27	8.39 ± 1.69
10 µg/ml	6.47 ± 2.02	2.67 ± 0.11
3 µg/ml	2.55 ± 1.03	2.55 ± 0.42

Table 4: CYP2B6 fold induction comparison between M. charantia and P. amarus

Test concentration	M. charantia	P. amarus
30 µg/ml	6.82 ± 3.68	4.81 ± 1.04
10 µg/ml	5.06 ± 2.02	3.30 ± 0.85
3 µg/ml	1.61 ± 0.11	2.36 ± 0.68

Table 5: CYP1A2 fold induction comparison between M. charantia and P. amarus

Test concentration	M. charantia	P. amarus
30 µg/ml	8.47 ± 3.87	7.89 ± 1.71
10 µg/ml	4.81 ± 3.22	5.54 ± 1.42
3 µg/ml	1.27 ± 0.16	2.45± 1.09

Increase in the activity of major CYP enzymes has been implicated in affecting the pharmacokinetics of several drugs which are the substrates of CYP isoforms. Herbs such as St. John's wort and foods such as grapefruit juice are examples of naturally occurring substances that can cause the reduction in drug-efficacy and/or produce life-threatening side effects when taken concomitantly with a variety of prescription drugs due to pharmacokinetic interactions[25]. In areas of the world with low access to medicinal solutions to diabetes, *M. charantia* has been said to be the most popular anti-diabetic herbal alternative[18]. *P. amarus* is used in various Asian countries as a treatment for hepatitis and jaundice[14]. It is also used as a liver tonic for maintenance of a healthy liver. Because of the widespread prevalence and use of these herbs, it is important to determine the likelihood for pharmacokinetic drug interactions if these herbs are concomitantly consumed with the prescription drugs.

In vitro studies utilizing human hepatocellular carcinoma cells were performed to analyze the PXR modulation and CYP induction capabilities of the two herbs. The results from a reporter gene assay to determine the modulation of PXR indicated that both herbs activated the PXR and increased its transcriptional activity at a level greater than two-fold compared to vehicle control. These results are consistent with the results of an earlier study performed by Fasinu et al (2017). Increased PXR activity levels have been linked with the induction of cytochrome P450 enzymes[23]. Due to this positive correlation, *M. charantia* and *P. amarus* were tested for their CYP induction capabilities. When analyzing CYP induction capabilities, *M. charantia* showed significant induction of all four CYP enzymes. At all three concentration levels, *M. charantia* significantly induced CYP3A4 implying that this herb is most likely to impact that pharmacokinetics of drugs

that utilize this pathway. *P. amarus* also significantly induced all four CYPs. At 30 µg/ml and 10 µg/ml, *M. charantia* induced all four enzymes at a greater strength than *P. amarus*.

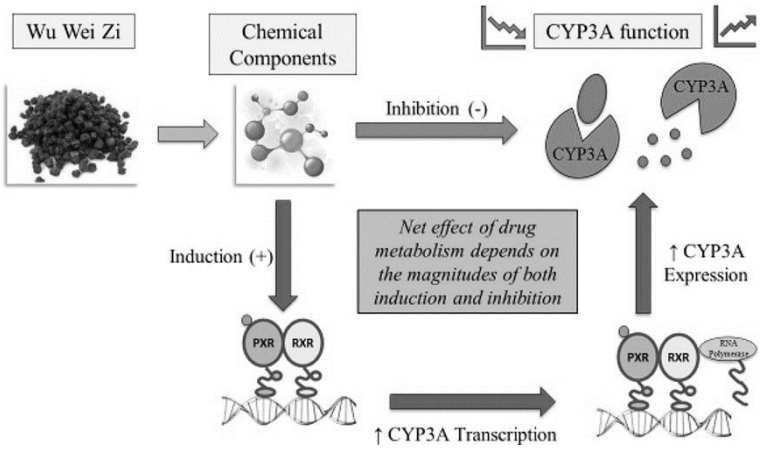
Both herbs significantly induced CYP3A4 similarly to St. John's wort which has been shown to reduce the effectiveness of drugs that are substrates of CYP3A4 such as alprazolam[24]. It is likely that drugs taken concomitantly with the tested herbal supplements could experience a reduction in efficacy and in turn will need dosage alterations to maintain desired treatment levels. However, these two herbs have also demonstrated CYP inhibition properties as reported earlier by Fasinu et al (2017). *P. amarus* had inhibitory effects on all four enzymes tested in this study while *M. charantia* did not inhibit CYP2B6.

A natural product with known CYP inhibitory effects is grapefruit juice[2]. Inhibitory effects are immediate and lead to an increase in drugs plasma concentration levels. In future studies, it is important to find out whether the inhibitory effects of the tested samples are reversible or not. This is important because the mechanism through which determines the length of the impact on the pharmacokinetics of other drugs. For example, grapefruit juice is partially irreversible thus the effects are relatively long-lasting[2]. It has been reported that when a drug is taken 12 hours after grapefruit juice ingestion, the rate that the drug enters systemic circulation doubles[30].

This capability of *M. charantia* and *P. amarus* to both inhibit and induce CYP isoforms is not contradictory. Conventional medicines are generated as isolated chemicals with a specific biochemical target in mind. Conversely, natural supplements have a unique composition of phytochemicals, thus enabling the herbs to impact different

biochemical targets and/or the same biochemical target in opposite manners[15]. In cases similar to that of *M. charnatia* and *P. amarus*, the net effect of the induction and inhibition of the same CYP isoform may be neutral and may not be significant enough to pose a clinically relevant risk. *In vitro* studies such as the one performed are only predictive of herb-drug interactions that can occur. In order to establish the final outcome, more advanced studies are warranted to establish an overall physiological effect.

Figure 13: Herb-drug interaction through induction or inhibition of CYP 450 Enzymes (Hogle et al, 2018)



4. CONCLUSION

There is a lack of literature analyzing the impact of two herbs, *M. charnatia* and *P. amarus,* on the ability to induce different CYP isoforms. In this study, PXR activity levels and CYP induction capabilities were analyzed as a predictor of herb-drug interaction capabilities. Both *M. charnatia* and *P. amarus* were found to increase PXR activity levels and induce CYP3A4, CYP1A2, CYP2B6, and CYP 2C9. This shows the potential of *M. charnatia* and *P. amarus* to alter drug pharmacokinetics. The *in vitro* studies performed are only predictive. Therefore, for a better understanding of the biochemical processes that will occur, further studies such as *in vivo* animal studies are recommended.

REFERENCES

1. Arslanlioglu, Sayat. “Momordica Charantia.” *TrekNature*, 22 Nov. 2008, www.treknature.com/gallery/photo188255.htm.
2. Bressler, Rubin. "Grapefruit juice and prescription drug interactions." *Geriatrics* 61.11 (2006).
3. Cattanach, Angela, and Siboniso Sibindi. “Warfarin, St John's wort and INR.” *Australian prescriber* vol. 39,2 (2016): 32-3. doi:10.18773/austprescr.2016.025
4. Chen, Jiezhong, and Kenneth Raymond. "Roles of Rifampicin in Drug-Drug Interactions: Underlying Molecular Mechanisms Involving the Nuclear Pregnane X Receptor." *Annals of Clinical Microbiology and Antimicrobials*, vol. 5, no. 1, 2006, pp. 3-3.
5. Cohrt, Karen. “The Lowdown on Transcriptional Reporters.” *Tempo Bioscience*, 1 Oct. 2018, www.tempobioscience.com/blog/?p=796.
6. Commissioner, Office of the. “FDA 101: Dietary Supplements.” *U.S. Food and Drug Administration*, FDA, 7 July 2015, www.fda.gov/consumers/consumer-updates/fda-101-dietary-supplements.
7. “Complementary, Alternative, or Integrative Health: What's In a Name?” *National Center for Complementary and Integrative Health*, U.S. Department of Health and Human Services, 2 Apr. 2019, nccih.nih.gov/health/integrative-health.

8. Conrad, Jim. “Naturalist Newsletter.” *PHYLLANTHUS AMARUS*, 11 Jan. 2019, www.backyardnature.net/yucatan/leafflwr.htm.

9. “Cytochrome P450 Drug Metabolism.” *DynaMed*, 30 Nov. 2018, www.dynamed.com/drug-review/cytochrome-p450-drug-metabolism#CYP3_FAMILY.

10. Ekor, Martins. “The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety.” *Frontiers in pharmacology* vol. 4 177. 10 Jan. 2014, doi:10.3389/fphar.2013.00177

11. Fahmi, Amr Mohamed et al. “Rifampin-warfarin interaction in a mitral valve replacement patient receiving rifampin for infective endocarditis: a case report.” *SpringerPlus* vol. 5 8. 4 Jan. 2016, doi:10.1186/s40064-015-1653-8

12. Fasinu, Pius S et al. “Modulation of Cytochrome P450, P-glycoprotein and Pregnane X Receptor by Selected Antimalarial Herbs-Implication for Herb-Drug Interaction.” *Molecules (Basel, Switzerland)* vol. 22,12 2049. 23 Nov. 2017, doi:10.3390/molecules22122049

13. Gates, Robert. “Phase I Drug Metabolism.” *Sigma*, www.sigmaaldrich.com/technical-documents/articles/biofiles/phase-i-drug-metabolism.html.

14. George, Annie, Jay K. Udani, and Ashril Yusof. "Effects of Phyllanthus amarus PHYLLPROTM leaves on hangover symptoms: a randomized, double-blind, placebo-controlled crossover study." *Pharmaceutical biology* 57.1 (2019): 145-153.

15. Hogle, Brett C., et al. "PXR as a mediator of herb–drug interaction." *Journal of food and drug analysis* 26.2 (2018): S26-S31.

16. Horn, John R, and Phillip D Hansten. “Get to Know an Enzyme: CYP1A2.” *Pharmacy Times*, 1 Nov. 2007, www.pharmacytimes.com/publications/issue/2007/2007-11/2007-11-8279.

17. Horn, John R, and Philip D Hansten. “Get to Know an Enzyme: CYP2C9.” *Pharmacy Times*, H&H Publications, 3 Mar. 2008, www.pharmacytimes.com/publications/issue/2008/2008-03/2008-03-8462.

18. Joseph, Baby, and D Jini. “Antidiabetic effects of *Momordica charantia* (bitter melon) and its medicinal potency.” *Asian Pacific Journal of Tropical Disease* vol. 3,2 (2013): 93–102. doi:10.1016/S2222-1808(13)60052-3

19. Karaman, Kevser, et al. "Molecular, Morphological and Biochemical Characterization of some Turkish Bitter Melon (Momordica Charantia L.) Genotypes." *Industrial Crops & Products*, vol. 123, 2018, pp. 93-99.

20. Kliewer, Steven A., and Timothy M. Willson. "Pxr, Car and Drug Metabolism." *Nature Reviews Drug Discovery*, vol. 1, no. 4, 2002, pp. 259-266.

21. Ma, Xiaochao, Jeffrey R. Idle, and Frank J. Gonzalez. "The Pregnane X Receptor: From Bench to Bedside." *Expert Opinion on Drug Metabolism & Toxicology*, vol. 4, no. 7, 2008, pp. 895.

22. Maartens, G., Decloedt, E., & Cohen, K. (2009). Effectiveness and safety of antiretrovirals with rifampicin: Crucial issues for high-burden countries. *Antiviral Therapy,* 14(8), 1039-1043. doi:10.3851/IMP1455

23. Manda, Vamshi K., et al. "PXR mediated induction of CYP3A4, CYP1A2, and P-gp by Mitragyna speciosa and its alkaloids*." Phytotherapy research* 31.12 (2017): 1935-1945.

24. Markowitz, John S., et al. "Effect of St John's wort on drug metabolism by induction of cytochrome P450 3A4 enzyme." *Jama* 290.11 (2003): 1500-1504

25. Mouly, Stéphane, et al. "Is the Clinical Relevance of Drug-Food and Drug-Herb Interactions Limited to Grapefruit Juice and Saint-John's Wort?" *Pharmacological Research*, vol. 118, 2017, pp. 82.

26. Murphy, Patricia A., et al. "Interaction of St. John's Wort with Oral Contraceptives: Effects on the Pharmacokinetics of Norethindrone and Ethinyl Estradiol, Ovarian Activity and Breakthrough Bleeding." *Contraception*, vol. 71, no. 6, 2005, pp. 402-408.

27. Patel, Jay Ram, et al. "Phyllanthus amarus: ethnomedicinal uses, phytochemistry and pharmacology: a review." *Journal of Ethnopharmacology* 138.2 (2011): 286-313.

28. Rashrash, Mohamed et al. “Prevalence and Predictors of Herbal Medicine Use Among Adults in the United States.” *Journal of patient experience* vol. 4,3 (2017): 108-113. doi:10.1177/2374373517706612

29. Regazzi, Mario, et al. "Treatment Optimization in Patients Co-Infected with HIV and Mycobacterium Tuberculosis Infections: Focus on Drug-Drug Interactions with Rifamycins." *Clinical Pharmacokinetics*, vol. 53, no. 6, 2014, pp. 489.

30. Rodríguez-Fragoso, Lourdes, and Jorge Reyes-Esparza. "Fruit/vegetable- drug interactions: Effects on drug metabolizing enzymes and drug transporters." *Chapter 1 (drug discovery);* 2013(Online: http://dx. doi. org/10.5772/48283) (2018).'

31. "St. John's Wort and Depression: In Depth." *National Center for Complementary and Integrative Health*, U.S. Department of Health and Human Services, Dec. 2017, www.nccih.nih.gov/health/st-johns-wort-and-depression-in-depth/.

32. Taxak, Nikhil, and Prasad V. Bharatam. "Drug Metabolism: A Fascinating Link between Chemistry and Biology." *Resonance*, vol. 19, no. 3, 2014, pp. 259-282.

33. Tebbens, Jurjen D., et al. "Mathematical Models in the Description of Pregnane X Receptor (PXR)-Regulated Cytochrome P450 Enzyme Induction." *International Journal of Molecular Sciences*, vol. 19, no. 6, 2018, pp. 1785.

34. Topletz, A. R., Dennison, J. B., Barbuch, R. J., Hadden, C. E., Hall, S. D., & Renbarger, J. L. (2013). The relative contributions of CYP3A4 and CYP3A5 to the metabolism of vinorelbine. *Drug Metabolism and*

Disposition: The Biological Fate of Chemicals, 41(9), 1651-1661. doi:10.1124/dmd.113.051094

35. "Treatment Highlights of Drug-Susceptible Tuberculosis Guidelines." *Centers for Disease Control and Prevention*, Centers for Disease Control and Prevention, 11 Aug. 2016, www.cdc.gov/tb/topic/treatment/guidelinehighlights.htm.

36. "Tuberculosis and HIV." *World Health Organization*, World Health Organization, 26 Mar. 2019, www.who.int/hiv/topics/tb/en/.

37. "Types of Complementary and Alternative Medicine." *Johns Hopkins Medicine*, www.hopkinsmedicine.org/health/wellness-and-prevention/types-of-complementary-and-alternative-medicine.

38. Watkins, Ryan E., et al. "The Human Nuclear Xenobiotic Receptor PXR: Structural Determinants of Directed Promiscuity." *Science*, vol. 292, no. 5525, 2001, pp. 2329-2333.