CHAPTER ONE

INTRODUCTION

1.1 Background

Herbs are taken as powdered products, standardized extracts, and tinctures or as beverages. Herbal beverages, wines, and teas, however, are quite popular worldwide due to convenience of use and perceived beneficial effects (Chan *et al*., 2010; Agbor *et al*., 2011; Samali *et al*., 2012). An herbal drink that is very popular in Nigeria and many parts of the world is the aqueous beverage of *Hibiscus sabdariffa* which is used for entertainment at social gatherings and taken as hot or cold drink (Kolawole and Maduenyi, 2004; Oboh and Elusiyan, 2004). The calyces of *Hibiscus sabdariffa* is cooked as vegetable, prepared as teas and beverages, jams and preservatives (Mahadevan *et al*., 2009). It is known as "Rosselle" in Mexico, "Karkade" in Germany, Egypt, Saudi Arabia and Sudan, "Sorrel" in the Caribbean and Latin America while in China it is called "Luo Shen Hua." In Nigeria, it is referred to as "Isapa pupa"or "*Zobo*" in the south-western part of the country and as "*Zoborodo*" in the northern part. In folk medicine, it is used for diverse ailments such as liver disease, hypertension, diabetes, kidney diseases, fever and as a laxative (Ali *et al.*, 2005; Mahadevan *et al.*, 2009).

The resurgence in the use of herbal medicines and herbs has led to increase in the concomitant use of herbs with drugs, with the belief that the potency of either the herb or the drug may be enhanced (Ioannides, 2002). However, the concomitant use of herbs with drugs has repeatedly given rise to various interactions which may not be beneficial to the user (Izzo, 2005).

A common practice in some parts of the world is the use of juices, sodas and sometimes herbal drinks such as the aqueous beverage of *Hibiscus sabdariffa* (*Zobo*) to administer

drugs (Huang and Lesko, 2004; Fakeye *et al.,* 2007a). Some herbal drinks have been known to interact with drugs. Grapefruit juice, a widely consumed drink and a known inhibitor of intestinal CYP3A4 isoform was reported to cause 0%, 60%, 230%, 1250% and 1400%, change in area-under-the-plasma concentration time curve (AUC) of pravastatin, cyclosporine, atorvastatin, lovastatin, and simvastatin, respectively (Yee *et al.,* 1995; Kantola *et al.,* 1998; Lilja *et al.,* 1999, 2000, 2004). Plasma levels of fexofenadine were decreased by juices of grapefruit, apple, and orange (Dresser *et al.,* 2002). Orange juice also decreased the peak plasma concentrations (C_{max}) of levofloxacin, gatifloxacin, and ciprofloxacin by 11%, 15%, and 41% respectively (Neuhofel *et al.,* 2002; Wallace *et al.,* 2003a, 2003b).

Many herbs and herbal preparations have been reported to cause herb-drug interactions some of which are potentially dangerous to the health of the patient, sometimes resulting in hospitalization. *Ginkgo biloba* causes spontaneous bleeding when coadministered with aspirin, ibuprofen, or warfarin (Bressler, 2005). Panax ginseng induces mania when used with phenelzine (Hu *et al.,* 2005), and St. John's Wort reduces plasma concentrations of midazolam, digoxin, and indinavir (Hu *et al.,* 2005).

Herb-drug interactions have been shown to occur when the pharmacokinetic profile of the drug is altered significantly by the coadministered herb. Many interactions involve inhibition of metabolizing enzymes and efflux transporters, resulting in increased systemic exposure and subsequent adverse drug reactions. However, most interactions during metabolism occur especially during phase 1 mediated by cytochrome P450 (CYP) isoforms. Cytochrome P450 isoforms are known to be responsible for the metabolism of approximately 70% of prescription drugs (Karyekar *et al.,* 2002). Many CYP isoforms such as CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 have been implicated in pharmacokinetic interactions in humans. Some herbs have been shown to inhibit or induce selected CYP isoforms. For example, acute usage of St. John's Wort (SJW) has little effect on major CYPs isoforms such as CYP1A2, CYP2D6, CYP2C9, CYP3A4 but chronic usage induces CYP3A4 (Wang *et al.,* 2001). Echinaceae, on the other hand, alters the action of CYP3A4 (Gorski *et al.,* 2004) while *Ginkgo biloba* extract induces CYP2C19 (Yin *et al.,* 2004). Some secondary metabolites such as flavonoid,

tannins, coumarins, alkaloids and anthocyanins, which are also present in the calyx of *Hibiscus sabdariffa*, have been shown to be inhibitors of cytochrome P450 enzymes (Obermeier *et al.*, 1995; Henderson *et al.*, 2000; Dreiseitel *et al.*, 2008; Kimura *et al.*, 2010; Sand *et al.*, 2010), Despite these numerous reports, there seems to be no study demonstrating the inhibitory activity of *Hibiscus sabdariffa* on CYP isoforms.

However, studies have shown that the calyx of *Hibiscus sabdariffa* possesses useful pharmacological properties such as strong antioxidant effects both *in vitro* and *in vivo* (Tseng *et al.*, 1996, Tseng *et al*., 1997; Akindahunsi and Olaleye, 2003; Essa *et al.*, 2006). Numerous studies in animal models and humans have also demonstrated that extracts and fractions of calyx of *Hibiscus sabdariffa* has antihypercholesterolaemic (El-Saadany *et al*., 1991; Chen *et al*., 2004; Carvajal-Zarrabal *et al*., 2005; Essa *et al*., 2006; Hirunpanich *et al*., 2006; Lin *et al*., 2007; Agoreyo *et al*., 2008; Gurrola-Díaz *et al*., 2010; Kuriyan *et al*., 2010), anxiolytic and immunomodulatory (Fakeye, 2008; Fakeye *et al*., 2008a), antinociceptive, antipyretic (Dafallah and Al-Mustafa, 1996) and antihypertensive activities (Faraji and Tarkhani, 1999; Onyenekwe *et al*., 1999; Odigie *et al*., 2003; Herrera-Arellano *et al*., 2004). These numerous pharmacological properties reported for *Hibiscus sabdariffa* make it a potential herb for self-medication in the treatment of diverse ailments and as an herbal beverage for its anxiolytic property (Fakeye *et al.*, 2008a).

1.2 Rationale for the study

Studies have shown that about 40% to 57% different patient groups use herbs along with their medications (Clement *et al*., 2005; Smith *et al*., 2010; Nordeng *et al*., 2011; Zhang *et al*., 2011). A study conducted by Djuv *et al*., (2013) showed that 45% of patients attending general practice co-used herbs with their medications and close to 40% of patients on anticoagulants also use herbs with their medications (Smith et al., 2010).Hospitalised patients likewise, supplement their drugs with herbs (Madsen *et al*., 2003; Yeh *et al*., 2006; Goldstein *et al*., 2007; Fakeye *et al*., 2008b; Fakeye *et al*., 2009), some of which could have potentially serious interactions. Most patients, however, do not disclose the herbs used to the physician (Robinson and McGrail, 2004; Fakeye *et al*., 2008b; Kennedy *et al*., 2008). This practice may predispose such people to herb-drug interactions, possibly modifying the outcome of therapy or resulting in untoward effects and toxic reactions.

Studies on the effect of co-administeration of aqueous beverage of *Hibiscus sabdariffa* with drugs (Mahmoud *et al*., 1994; Kolawole and Maduenyi, 2004; Fakeye *et al*., 2007a) have shown that the aqueous beverage of *Hibiscus sabdariffa* may possess the ability to elicit herb-drug interaction that may affect the outcome of therapeutic management of diseases. However, there seems to be no clear-cut mechanism by which the beverage elicited these interactions.

Hence, this study sought to evaluate the effect of *Hibiscus sabdariffa* on major cytochrome P450 isoforms responsible for the metabolism of over 70% of drugs and its effect on the pharmacokinetic parameters of coadministered drugs. Also, since the possibility of co-use of *Hibiscus sabdariffa* with an antilipidemic drug exists given the significant antilipidemic properties of *Hibiscus sabdariffa*, this study also evaluated the interactions between extracts of *Hibiscus sabdariffa* and an antilipidemic drug.

1.3 Aims and objectives

1.3.1 Overall objective

The main objective of this study was to evaluate the interactions of extracts and beverage of *Hibiscus sabdariffa* with selected drugs which are substrates of cytochrome P450 isoforms, with possible elucidation of mechanism of interaction.

1.3.2 Specific objectives

The specific objectives of this study were to evaluate:

i. The pattern of use of aqueous beverage of *Hibiscus sabdariffa* in a University community.

ii. *In vitro* inhibitory activity of ethanolic and aqueous extracts of *Hibiscus sabdariffa* on eight selected cytochrome P450 isoforms, and *in vivo* inhibitory activity of the aqueous beverage of *Hibiscus sabdariffa* on CYP1A2 and CYP2D6.

iii. The effect of aqueous beverage of *Hibiscus sabdariffa* on the pharmacokinetic of caffeine and simvastatin.

iv. Pharmacodynamic interactions between aqueous extract of *Hibiscus sabdariffa* and simvastatin (a CYP3A4 substrate).

1.4 Research Hypothesis

i. Aqueous beverage of *Hibiscus sabdariffa* is commonly used among staff and students of the University of Ibadan.

ii. Ethanolic and aqueous extracts of *Hibiscus sabdariffa* are inhibitors of CYP isoforms.

iii. Aqueous beverage of *Hibiscus sabdariffa* is an *in vivo* inhibitor of CYP1A2 and CYP2D6 isoforms.

iv. Aqueous beverage of *Hibiscus sabdariffa* alters the pharmacokinetic parameters of caffeine and simvastatin.

v. Addition of aqueous extract of *Hibiscus sabdariffa* to simvastatin improves lipid profile.

CHAPTER TWO

LITERATURE REVIEW

2.1 Drug interactions

Drug interactions are broadly divided into pharmacodynamics and pharmacokinetic interactions. Pharmacokinetic interactions may occur during absorption, distribution, metabolism or excretion. These interactions affect drug action by quantitative alterations, either increasing or decreasing the amount of drug available to elicit pharmacotherapeutic effect (Katzung *et al*., 2009; Tripathi, 2013).). Most clinically important drug-drug interactions (DDIs) and herb-drug interactions (HDIs) take place during the absorption or metabolism phase (Ioannides 2002). Pharmacodynamic interactions are qualitative and may result in either enhancement of drug effects (synergistic or additive actions) or antagonistic effects (Katzung *et al*., 2009; Tripathi, 2013).).

2.2 Types of drug interactions

Drug interactions could occur when two drugs are coadministered (drug-drug interactions). An example is the use of rifampicin with oral contraceptive or astemizole with erythromycin (Tripathi, 2013). Drug may interact with a disease condition (drug-disease interactions) leading to adverse effect of the drug, for example the use of paracetamol or the statins in hepatic disease (Tripathi, 2013).. Also concomitant use of herbs with conventional drugs may lead to herb-drug interactions such as the use of *Ginkgo biloba* with aspirin causing excessive bleeding in users (Hu *et al*., 2005; Izzo and Ernst 2012). There may be drug-food interactions when a drug is administered after a meal. For example, the absorption of griseofulvin and halofantrine/lumefantrine increases with meal (Tripathi, 2013). In drug-drug interactions (DDIs), two or more drugs are involved and one may quantitatively or qualitatively affect the level of the “object” drug in the body (Tripathi, 2013).

2.3 Magnitude of drug interaction effect

Drug-drug interactions (DDIs) are common in polypharmacy which may sometimes result from the management of multiple disease conditions in theelderly (Kelly, 2001; Juurlink *et al.*, 2003).. Some of these DDIs may lead to serious adverse drug reactions (ADR). The incidence of DDIs leading to ADRs is from 10% to 26%, depending on the population that is studied (Kelly, 2001; McDonnell and Jacobs, 2002; Juurlink *et al.*, 2003). Adverse drug reaction is estimated as one of the leading causes of death globally, resulting in increased hospitalization and a significant burden on the economy (Lazarou *et al*., 1998; McDonnell and Jacobs, 2002).

2.4 Mechanisms of drug interaction

2.4.1 Pharmacodynamic drug interactions

In pharmacodynamic interactions, the action, effect or the mechanism of action of a drug is affected by another drug. The site of interference may be at the level of receptors or at the level of physiological system or sometimes the action of the drug is changed because of changes in the homeostatic response (Boxtel *et al*., 2008). Pharmacodynamic drug interactions result in alteration of the response to one or both drugs without affecting their plasma concentrations (e.g. displacement from receptor binding sites). Generally, it could be broadly divided into synergistic, additive, potentiation or antagonizing effects (Katzung *et al*., 2009; Tripathi, 2013).

2.4.1.1 Additive, synergistic or potentiation interactions

An additive interaction is obtained when two drugs of similar pharmacology are concurrently administered and the resultant effect is the sum of their individual effects. A typical example is the excessive drowsiness occasioned by the use of two central nervous system depressants like phenylcyclidine and pentobarbital (Katzung *et al*., 2009). Potentiation means that drug-A, boosts the effects of drug-B, often by increasing the levels of drug-B in the blood. An example is the use of carbidopa to enhance the effect of L-dopa. Like synergism, this may be useful in cases in which the beneficial effects of drug-B are enhanced. However, the toxicities of drug-B may also be potentiated, leading to an increased level of side-effects. Synergistic interaction on the other hand is usually encountered when the combination of two drugs produces a pharmacological action

greater than the arithmetic sum of their individual effects. A classic example is the use of co-trimoxazole which contains trimethoprim and sulphamethoxazole, both bacteriostatic agents to achieve a bacteriocidal effect. Other examples include the combination of estrogen and progesterone in oral contraceptives, and hydrochlorothiazide and enalapril for enhanced antihypertensive effect (Tripathi, 2013).

2.4.1.2 Antagonistic drug interactions

There are two types of antagonistic pharmacodynamic drug interactions. These are receptor antagonism which are pharmacological in nature and non-receptor antagonism which is otherwise called non-pharmacologic antagonism.

2.4.1.2.1 Receptor antagonism – Competitive

Two drugs compete for the same receptor site by displacing each other depending on their affinity for the receptor. Competitive antagonism-based interactions are employed in the management of poison and drug overdose. Naloxone is used as an antidote in opiate poisoning. It competes with the same receptor site as the opiates by displacing it and preventing the drug from eliciting its pharmacological effect which may be greatly exaggerated as a result of overdose. Other examples include flumazenil in acute benzodiazepine overdose and atropine in organophosphorous compound poisoning (Tripathi, 2013).

2.4.1.2.2 Receptor antagonism – Noncompetitive

In non-competitive receptor antagonism, both the agonist and the antagonist bind to different active sites on the receptor moiety. The pair of diazepam and bicuculline or norepinephrine and phenoxybenzamine is an example of non-competitive antagonism. The blockade of α-receptors by phenoxybenzamine prevents vasoconstriction of peripheral blood vessels by endogenous catecholamines such as epinephrine. Bicuculline, on the other hand, a specific blocking agent of GABA-ergic receptors, when combined with diazepam, reduces the tranquilizing potential of diazepam (Ostrovskaya and Voronina, 1977; Grahame-Smith, 2016).

2.4.1.3 Non-receptor or Non-pharmacologic antagonism

In non-pharmacologic antagonism, the interaction between two concomitantly administered drugs is usually as a result of physical, chemical or physiological interactions. These interactions do not occur at the receptor sites.

2.4.1.3.1 Physiological antagonism

The two interacting drug affects the physiological system differently, sometimes their effect or action may be opposite in nature. In the combination of two diuretics hydrochlorothiazide and triamterene, the former causes potassium depletion while the later drug is a potassium sparing diuretic. Though they do not act at the same receptor site, however, they have opposing effect on the elimination of potassium ion. The loss of potassium ion by hydrochlorothiazide is minimized by the addition of triamterene (Möhrke *et al*., 1997; Katzung *et al*., 2009; Tripathi, 2013).

2.4.1.3.2 Chemical antagonism

This occurs when two coadministered drugs interact with each other by chemical reactions. Potassium permanganate and tannins are used in alkaloid poisoning. Potassium permanganate reacts with the unabsorbed alkaloids to form an unabsorbable material while tannins undergo a chemical reaction with the alkaloid to form insoluble alkaloidal tannate. Also in iron overdose, desferrioxamine chelates iron to form an unabsorbable complex (Katzung *et al.,* 2009; Tripathi, 2013).

2.4.1.3.3 Physical antagonism

Physical antagonistic interactions between two drugs could be in form of binding or adsorption; this is the basis for the use of activated charcoal to prevent the absorption of some poisons into the systemic circulation (Katzung *et al.,* 2009).

2.4.2 Pharmacokinetic drug interactions

Pharmacokinetic drug interactions are a consequence of altered levels of exposure to the drug or its metabolites through a combination of the following mechanisms (Piscitelli *et al.,* 2011; Palleria *et al.,* 2013).

2.4.2.1 Absorption effect

The mechanisms of drug absorption are passive diffusion, active transport, facilitated transport, ion-pair transport, and endocytosis (Ritschel, 1992). Some drugs have the capacity to alter the rate of absorption or the extent of absorption or both by affecting one of the processes of absorption. Change in the extent of absorption of more than 20% or greater than 1.2-fold is of considerable clinical significance (Gugler and Allgayer, 1990; Food and Drug Administration, 2012; European Medicines Agency, 2014). Some mechanisms of drug interactions affecting absorption are:

2.4.2.1.1 Changes in pH

The absorption rate of a drug by passive diffusion in the gastrointestinal tract especially the stomach and the small intestinal is affected by the dissolution rate and the solubility of the drug in the medium. Drugs that are weak bases are better absorbed in alkaline medium or at higher pH and weak acids are more absorbed at lower pH. Hence, a drug which alters the pH of the gastric medium may decrease or increase the absorption of another drug requiring opposing pH for its absorption. Unionized molecules are absorbed rather than ionized molecules. Thus, the solubility of a drug does not necessarily mean its complete absorption. Acidic drugs (weak acids - pKa = 3 – 8) are more soluble in alkaline medium but they are also more ionized in such medium, limiting the rate and possibly the extent of absorption of acidic drugs. This also hold true for weak bases (pKa = 5 – 11) which have reduced absorption in acidic medium or low pH (Rodgers and Rowland, 2006). Interaction between two drugs may become clinically important if the extent of absorption of one drug is significantly affected by another drug as a result of its effect on the pH of the medium. Some drugs are known to raise the gastric pH mildly or appreciably. Examples of these drugs are antacids which raise the gastric pH slightly for about 30 minutes to 2 hours by 1 - 2 points (Fisher *et al.*, 1997). Histamine H_2 receptor antagonists and proton pump inhibitors have the ability to raise and maintain the gastric pH above 5.0 in dose dependent fashion (Burget *et al.*, 1990; Armstrong, 2004; Katz *et al.*, 2013). The coadministration of H_2-receptor antagonist, antacids and proton pump inhibitors with basic drugs like azole antifungals and β-lactam antibiotics alters their rate and extent of absorption (Gugler and Allgayer, 1990; Sadowski, 1994; Kanda *et al.*, 1998; Ogawa and Echizen, 2011).

2.4.2.1.2 Chelation and adsorption

Formation of insoluble complexes by chelation when two drugs are coadministered may prevent the dissolution of one of the drugs in gastric fluid and its subsequent absorption. The quinolones are major culprit in this type of drug interaction when divalent and trivalent cations such as ferrous sulphate, sucralfate, certain buffers, magnesium and aluminium containing antacids are coadministered. These cations form complexes with the 4-oxo and 3-carboxyl groups of the quinolones, and leads to clinically significant decreases in the quinolone area under the concentration–time curve (AUC) by 30 to 50% (Lomaestro and Bailie, 1991; Damle *et al.,* 2002).

2.4.2.1.3 Changes in gastric emptying and intestinal motility

Acid-labile drugs like erythromycin and penicillins are easily broken down in the stomach when gastric resident time is extended (Kristensen, 1976; Atkinson and Huang, 2012). Changes in gastric emptying and gastrointestinal motility may have significant effects on the absorption of some drugs, especially drugs that are absorbed in the small intestine. Reduced gastrointestinal transit time caused by the coadministration of drugs like cisapride, metoclopramide, and domperidone, may affect the extent of absorption of poorly soluble drugs or drugs that are absorbed in a limited area of the intestine (Tonini, 1996).

2.4.2.1.4 Effects of intestinal blood flow

Drug with vasoconstrictive properties may theoretically reduce blood flow to the intestine and consequently affect the absorption of other drugs especially lipophilic substances. There is a dearth of evidence on the clinical importance of this type of drug interactions (Katzung *et al.,* 2009; Tripathi, 2013).

2.4.2.1.5 Changes in active and passive transport

Many transporter proteins in the small intestine have been identified and their inhibition by drugs like quinolones have also been established (Tsuji and Tamai, 1996; Omkvist *et al.,* 2010; Estudante *et al.,* 2013; König *et al.,* 2013). This represents another way by which drug interaction may occur. Some drug can modify intestinal epithelial tight junctions and affect paracellular drug absorption. A well-known example is

acetaminophen or paracetamol. Caco-2 cell lines have been used to demonstrate this (Kamath et al; Deli, 2009).

2.4.2.1.6 Changes in presystemic clearance

Cytochrome P450 3A4/5 and P-glycoprotein are present in the luminal surface of the intestine. Cytochrome P450 3A4/5 are responsible for the metabolism of drugs in the small intestine while p-glycoprotein is an efflux protein which ejects unchanged drugs from the enterocytes into the lumen (Hillgren *et al*., 2013). The activity of cytochrome P450 and p-glycoprotein may affect the rate and extent of absorption of drugs which are substrate of these enzymes and transport protein.

2.4.2.2 Distribution effect

Drug interactions resulting from changes in the distribution of a drug are those that primarily affect or cause changes in plasma protein binding of the drug. The resultant effect of these drug interactions are sometimes overestimated. This is mostly due to *in vitro-in vivo* extrapolations, neglecting other physiological changes after drug displacement from protein binding sites (Smith *et al.,* 2010). The transient increase in the free drug after its displacement from plasma protein may become clinically insignificant if the displacement is immediately followed by redistribution and excretion of the drug (Smith *et al.,* 2010).

2.4.2.2.1 Protein Binding and Displacement

The major plasma protein in the human blood is albumin which has the capacity to bind to both basic and acid drugs. Erythromycin, a basic drug binds to albumin weakly at numerous binding sites than sulphonamide and doxycycline which are acidic drugs. The latter drugs bind strongly to fewer binding sites on the albumin (Zeitlinger *et al.,* 2011). It has also been shown that basic drugs bind more to α-1-acid glycoprotein (Zeitlinger *et al.,* 2011).

Drugs that are 80 - 90% bound to plasma proteins are more likely to elicit clinically significant drug interactions when displaced from plasma protein binding sites. With this type of drugs, a small displacement in the binding sites may lead to relatively large free unbound drugs in the plasma. The increased plasma drug concentration of free drugs after

displacement is immediately redistributed to other tissues in the body especially drugs with large volume of distribution. The presence of free drugs at the metabolism and excretion sites may lead to increased elimination of the drug. Rowland (1980) showed that majority of clinically important drug interactions resulting from plasma protein binding displacement often involve another secondary unrecognised mechanism of interaction (Smith *et al.,* 2010).

2.4.2.3 Drug metabolism effect

The major route of drug elimination from the body is enzymatic biotransformation which is divided into phase I and phase II. Phase I involves the transformation of xenobiotics into more hydrophilic molecule by the introduction of functional groups through oxidative reactions catalysed mainly by cytochrome P450 (CYP) (Klaassen *et al.,* 2013). Phase II reactions are mainly conjugation reactions by a diverse group of conjugating enzymes, like uridine glucuronosyl transferases (UGTs) and glutathione-S-transferases (Parkinson, 2001).

The liver plays a major role in the metabolism of xenobiotics and contains numerous metabolic enzymes. Most drugs absorbed in the gastrointestinal tract via the hepatic portal system are first metabolised by the cytochrome P450 isoforms present in the small intestine and in the liver (George and Shand, 2013). Drugs that undergo extensive metabolism by the liver have low oral bioavailability.

2.4.2.4 Excretion effect

Elimination of drugs through the kidneys usually involves glomerular filtration, tubular secretion and reabsorption. Several mechanisms of drug–drug interactions may take place in the kidneys (Bonate *et al.,* 1998; Knights *et al*., 2013)

2.4.2.4.1 Glomerular filtration

Glomerular filtration rate may fluctuate as a result of changes in renal blood flow, cardiac output, and extent of protein binding (van Ginneken and Russel, 1989). An increase in free drugs of highly protein bound drugs can lead to increase glomerular filtration and consequently an increase in the elimination rate of the drug (Piscitelli *et al.,* 2011).

Most organic cations and anions excreted via the kidney through renal tubular secretion could undergo drug-drug interactions by competing for tubular active transport system. Probenecid prevents tubular secretion of penicillins when coadministered, minimising the excretion of penicillins. This was used to sustain systemic concentration of penicillin for its antibacterial activity (Finch *et al.,* 2010). Other examples of drugs displaying clinically significant drug-drug interaction through renal tubular secretion are sulfonamides, penicillins, and zidovudine (Finch *et al.,* 2010).

P-glycoprotein is also involved in drug-drug interaction at the apical membrane of the proximal convoluted tubule. It is responsible for transporting large number of drugs into the urine. Inhibition of p-glycoprotein could lead to increased plasma concentration of drugs like the quinolones, macrolides, and azole antifungals which have high affinity for renal p-glycoprotein and can potentially contribute to significant drug interactions (Lee and Kim, 2004).

2.4.2.4.2 Tubular Reabsorption

Renal reabsorption of drugs could either be through passive diffusion or active transport processes. It is, generally known, that unionized drugs may be reabsorbed in the kidney tubules through passive diffusion process. An alteration in the urinay pH could alter the reabsorption of weak acids or bases (Ariens, 2012). Alkaline urinary pH will encourage the ionization of weak acids and increase their elimination while same pH may cause increased reabsorption of weak bases and reduce their elimination. This interaction may occur theoretically but often times they are not of clinical importance since the alteration in urinary pH does not have large effect on the plasma half-life of the drug (Ariens, 2012).

2.5 Significance of drug interactions

Most drug-drug or drug-herb interactions are first evaluated *in vitro.* However, correlations between the *in vitro* and *in vivo* findings are not always absolute. Also significant findings in clinical trial are not always clinically important. A less than 20% change in the pharmacokinetic parameters of a drug with narrow therapeutic window would be clinically important while same 20% change in pharmacokinetic parameters of a drug with wide therapeutic window would be of little or no clinical significance (Food and Drug Administration, 2012; European Medicines Agency, 2014).

Clinically significant drug-drug interactions between rifampicin and drugs like warfarin, cyclosporine and oral contraceptives have been documented to result in subtherapeutic levels of these drugs as a result enzyme induction (Piscitelli *et al*., 2011). Reduced plasma concentrations of the drugs may lead to treatment failure. Conversely, the reduction of peak plasma concentration (C_{max}) or AUC of antibiotics by another drugs or environmental influences can result in therapeutic failure and possibly an increase in the development of resistance.

Some drugs like ketoconazole have been used for a number of years to inhibit the metabolism of oral cyclosporine by approximately 80%. This ensures that lower doses of cyclosporine is used reducing the cost of therapy as well as the rates of rejection of organ transplant and infection (Keogh *et al.,* 1995; Jones, 1997). Beneficial and detrimental pharmacodynamic drug interactions also exist. The use of lower concentrations of two synergistic antibacterials to reduce the toxicity of each and to elicit same pharmacological effect has been advocated (Kempf *et al.,* 1997; Safdar *et al*., 2004).

2.6 Cytochrome P450 enzyme systems

The Cytochrome P450 is a superfamily of heme-containing mono-oxygenase enzymes (Figure 2.1 and 2.2). They play a major role in the oxidative, peroxidative, and reductive metabolism of a varied group of substances (Yan and Caldwell, 2001). Cytochrome P450 catalyses the metabolic reactions that involves the addition of an oxygen atom to the substrate and the other oxygen atom is converted to water with reducing equivalents derived from Nicotinamide Adenine Dinucleotide Phosphate (NADPH) (Guengerich, 2003; Klaassen *et al*., 2013). The CYP enzymes are found mostly in the microsomes and are responsible for over 80% of the metabolism of all drugs (Pelkonen, 2002; Wienkers and Heath, 2005).

Cytochrome P450 superfamily is divided into families with 18 families currently known and 42 known subfamilies in humans (Lewis, 2004; Nelson *et al.,* 2004). The CYP families 1 to 3 are involved in the metabolism of xenobiotics while the others are involved in the synthesis of endogenous substances like fatty acids, hormones and bile acids, as shown in Figures 2.1 and 2.2 (Nebert and Russell, 2002).

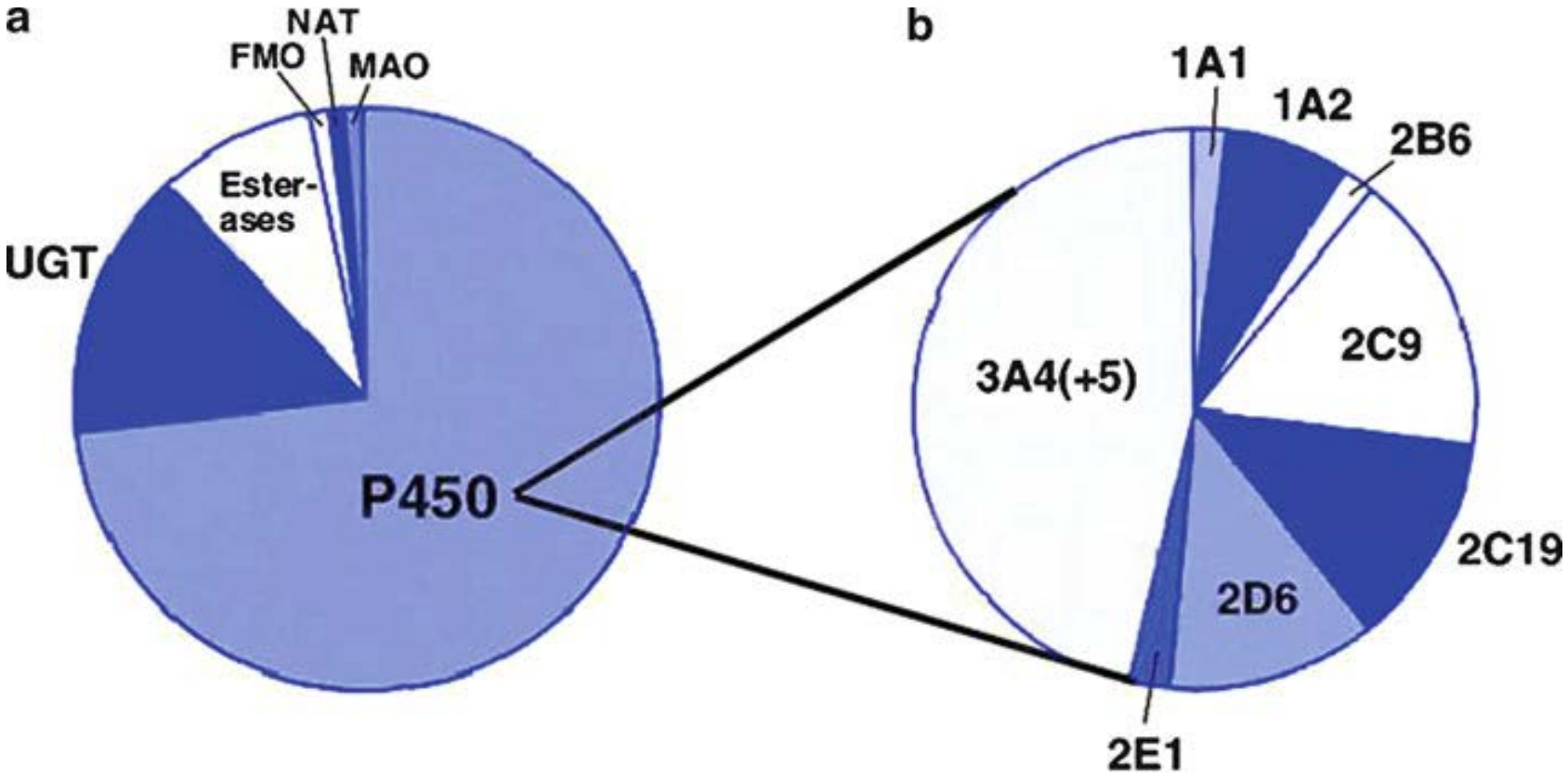


Fig 2.1: Contributions of enzymes to the metabolism of top 200 prescribed drugs. (a) Fraction of metabolic clearance catalyzed by various human drug-metabolizing enzymes. (b) Fractions of P450 oxidations on drugs catalyzed by individual P450 enzymes. (Williams *et al*., 2004; Guengerich, 2007).

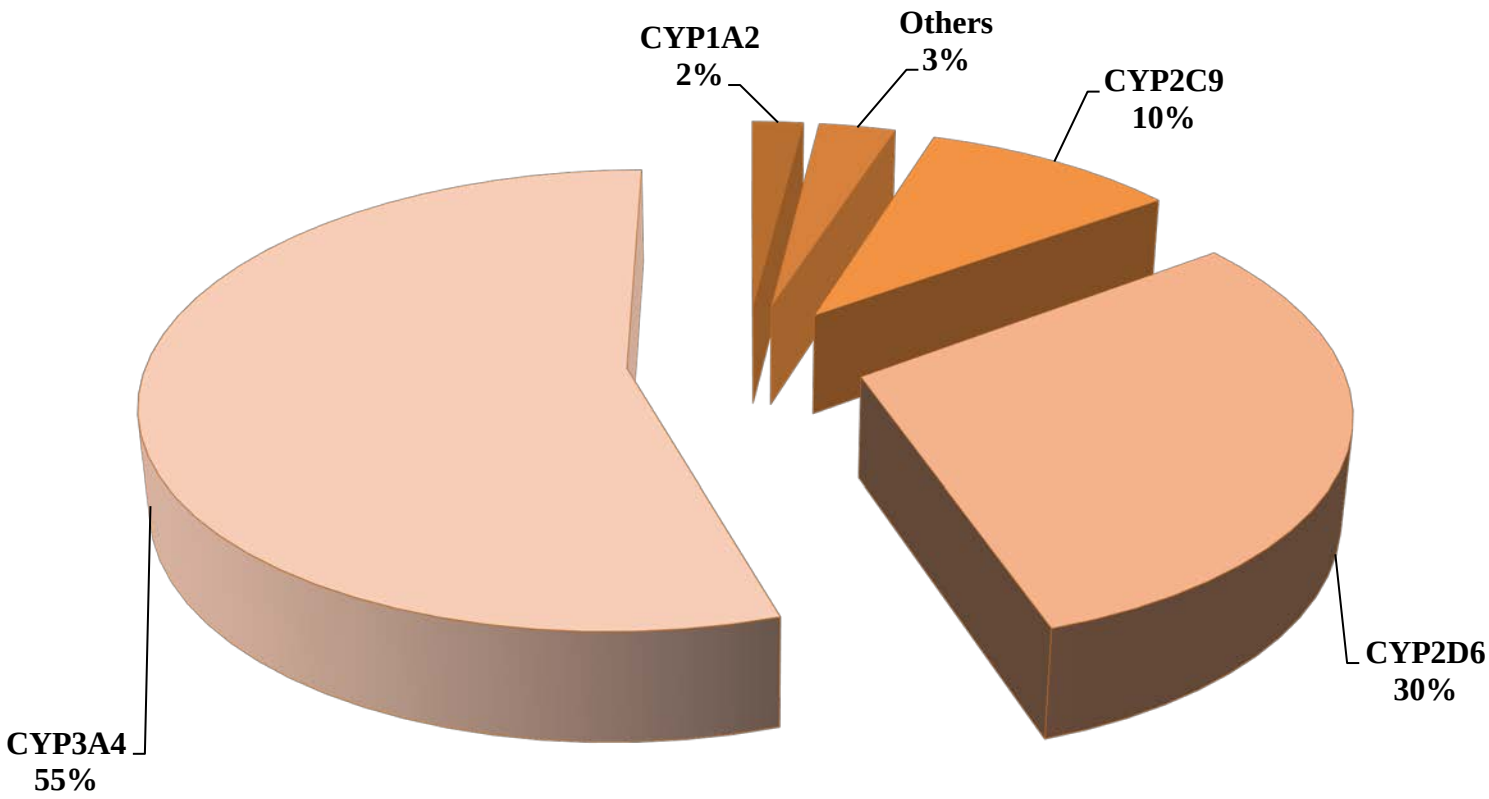


Fig 2.2: Individual contribution of each cytochrome P450 isoform to drug metabolism and their distribution

There is inter-individual variation in the contents and expression of CYP isoforms as a result of genetic variation, gender differences, environmental factors and some disease state. These factors are responsible for the variation of individual differences in the rate and extent of metabolism of some xenobiotics and the pathway of the metabolic process. External factors, like food, alcohol, coadministered drugs, and cigarette smoking may induce or inhibit one or more CYP isoform leading to changes in the rate at which the affected CYP isoform metabolises drugs (Kalow, 2001; Pacifici and Pelkonen, 2001).

Inter-individual variation in the expression of CYP isoforms due to genetic variation leads to polymorphic expression of specific CYP isoforms in the population (Daly, 2004; Solus *et al.,* 2004). This results in variability in the expression, activity, function, and stability of the enzyme. Polymorphic expression of CYP isoforms has been implicated in idiosyncratic ADRs (Rodrigues and Rushmore, 2002).

2.6.1 Induction of cytochrome P450 enzymes

Induction of cytochrome P450 enzymes results from an increased synthesis of CYP isoforms that is associated with exposure to drugs (Meyer and Rodvold, 1996, Ioannides, 2002). This may occur when two drugs are coadministered and one of the drugs causes the biotransformation of the other drug via a known metabolic pathway or through an alternate metabolic pathway. Generally, CYP isoform inducers are specific for each CYP isoform or the CYP family (Meyer and Rodvold, 1996, Ioannides, 2002). Sometimes a drug can induce its own biotransformation in addition to that of other drugs. Though, the result of an induction of a CYP isoform can manifest within two days of stimulation of the enzyme, it usually takes about a week for the complete synthesis of new CYP isoforms and for the full effect of the induction to materialise (Døssing *et al*., 1983; Zhou, 2008). The time taken for the onset and offset effect of inducers is dependent on the plasma concentration of the inducer and the half-life of the production and degradation of the CYP isoform involved (Døssing *et al*., 1983; Zhou 2008).

2.6.2 Inhibition of cytochrome P450 enzymes

The inhibition of CYP isoforms has led to the withdrawal of some drugs from the market due to the consequences of DDIs. These drugs include astemizole, terfenadine, and

cisapiride (Lasser *et al.,* 2002). The inhibition of the metabolism of a compound can lead to increased bioavailability of the compound or drug especially if it is prone to extensive first-pass metabolism. Also, drugs whose elimination is dependent on metabolism by CYP isoform could have its level in the body increased and consequently decreased elimination as a result of the inhibition of the CYP isoform responsible for its metabolism.

Drugs which are metabolised by a single CYP isoform or have a single metabolic pathway may have increased serum concentration, steady state, and toxicity with possibility of non-linear pharmacokinetics as a result of the saturation of the enzymic biotransformation (Lasser *et al.,* 2002). The inhibition of CYP isoforms responsible for the metabolism of prodrugs may lead to sub-therapeutic level of the drug, loss of activity, therapeutic failure, increased side effects and toxicity.

2.6.2.1 Classification of CYP inhibition

Cytochrome P450 enzyme inhibition can be classified into two, namely, irreversible inhibition (mechanism-based inhibition) and reversible. The irreversible type of inhibition requires that the inhibitor be modified or bio-transformed, while the reversible inhibiton is the most common type and could be competitive, uncompetitive, non-competitive and mixed-type inhibition (Ogilvie and Ruedy, 1967; Liljefors *et al*., 2002).

2.6.2.1.1 Irreversible inhibition (Mechanism-based inhibition)

Mechanism-based inhibitor act by forming strong bond or complexes with the enzyme. They are only terminated when new enzymes are synthesised, therefore the inhibition lasts long. Sometimes, the complex that is formed with the heme group on the CYP isoform completely inactivates the isoform. This form of inhibition is sometimes referred to as “suicide inhibition” (Kent *et al.,* 2001). Examples of mechanism-based inhibitors include furafylline which inhibits CYP1A2 and gestodene which inhibits CYP3A4 (Guengerich, 1990; Back *et al.,* 1991; Kunze and Trager, 1993).

2.6.2.1.2 Reversible inhibition

Reversible inhibition occurs when the inhibitor forms a weak bond with the active site of the enzyme. This bond is easily broken and is responsible for the short duration of action

of reversible inhibitors (Ogilvie *et al.,* 2008). Most drugs which inhibit enzymes for their activity are reversible inhibitors such as Angiotensin Converting Enzyme (ACE) inhibitors and 3-hydroxy 3-methylglutaryl Coenzyme-A (HMG-CoA) reductase inhibitors (Liljefors *et al.,* 2002). In competitive reversible inhibition, the substrate and the inhibitor compete for the same active site on the enzyme (examples are sulfanilamide, cycloserine, L-fluoroalanine, ethotrexate, and allopurinol). In non-competitive reversible inhibition, both the inhibitor and the substrate binds to different active sites on the enzyme moiety, examples include potassium cyanide and malonate ion while in the uncompetitive reversible inhibition the inhibitor binds to the enzyme-substrate complex. The mixed-type inhibition combines the characteristics of both competitive and non-competitive reversible inhibition (Liljefors *et al.,* 2002; Ogilvie *et al.,* 2008).

2.7 Human hepatic CYP isoforms involved in drug biotransformation

Many CYP isoforms have distinct selectivity towards probe substrates and inhibitors. Most of the CYP isoforms metabolises specific probe substrates and are inhibited by specific inhibitors. However, this selective property of the CYP isoforms may become overlapping such that a drug may be a probe substrate for more than one CYP isoforms and also an inhibitor may have the capacity to inhibit more than one isoforms. Table 2.1 shows the commonly used probe substrates and inhibitors for each CYP isoform.

2.7.1 CYP1 family

The members of this family include CYP1A1, CYP1A2 and CYP1B1. Of these three, CYP1A2 is the only member of this family that is highly concentrated in the liver. Cytochrome P4501A1 is commonly found outside the liver while CYP1B1 is ubiquitous (Shimada *et al.,* 1996; Ding and Kaminsky, 2003). Members of this family do not have pseudogenes and they are induced by 2, 3, 7, 8 tetra chlorodiabenzo-p-dioxin (TCDD), Polycyclic Aromatic Hydrocarbons (PAHs), and smoking (Nelson *et al.,* 2004; Shimada and Fujii-Kuriyama, 2004). Aside from detoxification, the members of this family are known to produce chemical carcinogens through the metabolism of PAHs, and compounds containing aromatic amines (Shimada and Fujii-Kuriyama, 2004).

Table 2.1 Summary of human hepatic drug metabolising CYP enzymes and their selected probe substrates and inhibitors used *in vitro* and *in vivo* studies

CYP	% in liver*	Substrate	Inhibitor	Other characteristics
1A2	2	Ethoxyresofin	Furafylline	Inducible
		Melatonin	Fluvoxamine	Polymorphic
		Phenacetin		
		Caffeine		
2A6	2	Coumarin	Tranylcypromine	Inducible
		Nicotine		Polymorphic
2B6	2-4	Bupropion	Thio-tepa	Inducible
		Cyclophosphamide	Ticlopidine	Polymorphic
		Efervirenz		
2C8	1	Paclitaxel	Monteleukast	
		Amodiaquine	Quercetin	Polymorphic
		Rosiglitazone		
2C9	10	Diclofenac	Sulphaphenazole	Inducible
		S-Warfarin		Polymorphic
		Losartan		
		Tolbutamide		
2C19	5	Omeprazole	Fluconazole	Polymorphic
		S-Mephenytoin		
		Proguanil		
2D6	2-3	Bufuralol	Quinidine	Polymorphic
		Propranolol	Paroxetine	
		Debrisoquine		
		Dextromethorphan		
2E1	2-4	Chlorzoxazone	Pyridine	Inducible
		Ethanol	Disulfiram	
3A4	45-50	Midazolam	Ketoconazole	Inducible
		Simvastatin	Itraconazole	
		Erythromycin		
		Testosterone		
		Nifedipine		

* The relative amount of hepatic CYP protein varies highly among people. Values are based on a meta-analysis. Data adapted from Bertz and Granneman, (1997); Pelkonen *et al.*, (1998), Pelkonen *et al.*, (2000); Rendic, (2002); Lewis, (2003); Ingelman-Sundberg, (2004); Pelkonen *et al.*, (2005); Totah and Rettie, (2005).

2.7.1.1 CYP1A2

Though CYP1A2 is the only member of the CYP1 family mostly found in the liver, other studies have shown that it is equally expressed in the lungs along with CYP1A6 (Zhou *et al*., 2004, Zhou *et al*., 2009). Cytochrome P450 1A2 is responsible for the metabolism of important chemicals and drugs such as caffeine, phenacetin, theophylline, clozapine, melatonin and tizanidine (Fang *et al*., 1998; Facciolá *et al*., 2001; Granfors *et al*., 2004a; 2004b). There are several potent inhibitors of CYP1A2, among these are furafylline, fluvoxamine, ciprofloxacin, and contraceptives (Sesardic *et al*., 1990; Pollock *et al*., 1999;).

Cytochrome P_{450} 1A2 is induced by cigarette smoking, cruciferous vegetables, and charcoal-grilled food (Murray *et al.,* 2001; Larsen and Brøsen, 2005). The effect of smoking on the metabolism of atypical antipsychotics by CYP1A2 leading to poor outcome in the treatment of schizophrenia has been studied quite extensively. This has led to dose adjustment in the treatment of schizophrenia (Bozikas *et al.,* 2004; de Leon, 2004).

2.7.2 CYP2 family

The members of this family include CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP2E1. These isozymes are diverse in their tissue expression and probe substrate specificity. The most studied CYP isoforms in this family is 2D6. Phenotypic expression of these isoforms is common among CYP2C9, CYP2C19 and CYP2D6.

2.7.2.1 CYP2A6

Quantitatively, CYP2A6 has a low expression in the liver compared to other CYP isoforms. Its probe substrate includes coumarin (Pelkonen *et al.,* 2000) and nicotine (Hukkanen *et al.,* 2005). This enzyme is known to bioactivate aflatoxin B1 and nitrosamines to some extent (Pelkonen *et al.,* 2000). Some of the potent inhibitors of this enzyme are tranylcypromine and methoxsalen (Draper *et al.,* 1997). Cytochrome P450 2A6 has been reported to induce metabolism of antiepileptics such as carbamazepine and phenytoin (Pitarque *et al.*, 2005).

2.7.2.2 CYP2B6

Previously, CYP2B6 was thought to occur in small quantity in the liver and has minor influence on the metabolism of xenobiotics. Research into its activity was limited by the lack of specific probe substrate, and potent inhibitors (Wrighton and Stevens, 1992). It has been reported that CYP2B6 is about 10% of total liver CYP content and inter-individual variability in the expression of the enzyme exists (Lang *et al.,* 2001). This enzyme plays a major role in the metabolism of bupropion, cyclophosphamide, efavirenz and ketamine (Faucette *et al*., 2000; Huang *et al*., 2000; Court *et al*., 2001; Hijazi and Boulieu, 2002). Cytochrome P_{450} 2B6 is involved in the bioactivation and detoxification of some precarcinogens (Smith *et al*., 2003). Potent inhibitors of CYP2B6 include the antiretroviral drugs like ritonavir, efavirenz, and nelfinavir (Hesse *et al.,* 2001).

2.7.2.3 CYP2C8

The importance of CYP2C8 for drug metabolism has been elucidated (Totah and Rettie, 2005) and the probe substrates include amodiaquine, paclitaxel, cerivastatin, repaglinide and rosiglitazone (Backman *et al*., 2002; Niemi *et al*., 2003). A number of functional *CYP2C8* polymorphisms have been identified (Niemi *et al.,* 2003) and associated with increased risk of acute myocardial infarction (Yasar *et al.,* 2004) and cerivastatin induced rhabdomyolysis (Ishikawa *et al.,* 2004). Potent CYP2C8 inhibitors include quercetin, montelukast, zafirlukast (Kim *et al*., 2005; Walsky *et al*., 2005).

2.7.2.4 CYP2C9 and CYP2C19

This enzyme is the predominant member of the CYP2C isoforms subfamily and is responsible for the metabolism of clinically important drugs like fluoxetine, fluvastatin, glimepiride, glipizide, losartan, *S*-warfarin, and several non-steroidal anti-inflammatory drugs - NSAIDs (Sullivan-Klose *et al.,* 1996; Rettie and Jones, 2005). Among the recognised inhibitors of CYP2C9 are gemfibrozil (Wen *et al.,* 2001) and amiodarone (Heimark *et al.,* 1992). Cytochrome P450 2C9 is phenotypically expressed and this explains the inter-individual variation in the metabolism of drugs especially those with narrow therapeutic window like S-warfarin (Kirchheiner and Brockmöller, 2005). Cytochrome P_{450} 2C19 is involved in the metabolism of important drugs like omeprazole, diazepam, citalopram, amitriptyline (Jiang *et al.,* 2004), proguanil and phenytoin (Bajpai

et al., 1996; Komatsu *et al.,* 2000). There is no selective inhibitor for this enzyme; however omeprazole and fluconazole are sometimes used (Niwa *et al.,* 2005).

2.7.2.5 CYP2D6

This is the most studied CYP isoform with respect to pharmacogenetics. The genetic polymorphism in this isoform is responsible for the inter-individual variation in the expression and activity of this enzyme which ranges from complete lack leading to toxic reaction or adverse drug reactions and excessive expression causing treatment failure (Bertilsson *et al.,* 2002; Ingelman-Sundberg, 2005). A study by Rau *et al*., (2004) showed that *CYP2D6* genotype is associated with the occurrence of adverse effects and clinical nonresponse in psychiatric patients treated with CYP2D6-dependent antidepressants. Environmental factors have no influence on CYP2D6 unlike other types of CYP isoforms; however pregnancy has been shown to increase the rate of CYP2D6-mediated metabolic reactions (Ingelman-Sundberg, 2005).

2.7.2.6 CYP2E1

The isoform CYP2E1 is the only member of its family and it metabolises only a few drugs. Chlorzoxazone is the only known probe substrate for this enzyme (Peter *et al.,* 1990). Cytochrome P450 2E1 is important in the bioactivation of several industrial solvents, metabolism of alcohol, paracetamol-related hepatotoxicity and activation of chemical carcinogens and production of free radicals causing injuries (Caro and Cederbaum, 2004; Rumack, 2004; Gonzalez, 2005). Potent inhibitors of CYP2E1 include pyridine and disulfiram (Kharasch *et al.,* 1993; Hargreaves *et al.,* 1994).

2.7.3 CYP3 family

Cytochrome P_{450} 3 is considered the most important CYP family; it represents about 30% of hepatic CYP content and is the most important CYP isoform as the metabolism of majority of clinicallyused drugs depends on the members of this family especially CYP3A4 and CYP3A5. This family contains one subfamily comprising three functional proteins: CYP3A4, CYP3A5, and CYP3A7, and one pseudoprotein, CYP3A34 (Ingelman-Sundberg, 2005). These enzymes have overlapping metabolic specificities and their tissue expression patterns differ. CYP3A5 is mainly expressed extrahepatically

while CYP3A7 is mainly expressed in embryonic, fetal, and newborn livers, where it is the predominant CYP form (Kitada and Kamataki, 1994; Hakkola *et al.*, 2001).

2.7.3.1 CYP3A4

Cytochrome P_{450} 3A4 is the major CYP isoform in the liver and the intestine, and plays an important role in the metabolism of about 50% of xenobiotics (Bertz and Granneman, 1997; Paine *et al.,* 2006). The active site of CYP3A4 is very large and could accommodate many small molecules, thus the enzyme could undergo multiple conformations due to substrate binding on these active sites. Some of the probe substrate of this enzyme are clinically important drugs like paracetamol, erythromycin, nifedipine, midazolam, simvastatin, and quinidine including endogenous substances like testosterone, progesterone, androstenedione, and bile acid (Rendic, 2002; Patki *et al.*, 2003). Hence, changes in the activity of CYP3A4 could lead to notable drug-drug interactions and adverse effects. The enzyme is also involved in the bioactivation of aflatoxin B1 and PAHs (Aoyama *et al.,* 1990). Cytochrome P_{450} 3A4 is susceptible to inhibition because of its low substrate selectivity. Some of the inhibitors are ketoconazole, itraconazole, clarithromycin, erythromycin, verapamil, diltiazem, and several herbal and food constituents, e.g. grapefruit juice and bergamottin (Fujita, 2004; He and Edeki, 2004).

Fifty percent inhibitory concentration, IC_{50}, for CYP3A4 inhibitors vary depending on the substrate that is used. Thus, more than one probe substrate is required in inhibition study of this CYP isoform (Galetin *et al.,* 2003). Strong inhibition of CYP3A4 may lead to ADRs, but in clinical practice, the inhibition of this enzyme has been used positively in the "boosting effect" of ritonavir in the treatment of human immunodeficiency virus (HIV) infection with other protease inhibitors (Plosker and Scott, 2003). Cytochrome P_{450} 3A4 is induced by wide variety of compounds e.g phenobarbital, rifampicin, dexamethasone, phenytoin, carbamazepine, and St. John's wort (Pelkonen *et al.,* 1998; Luo *et al.,* 2004).

2.8 Approaches to study cytochrome P450 enzymes, drug metabolism, and drug interactions

During the past years, several *in vitro* and *in silico* approaches for CYP screening purposes have been developed and taken into routine use within industry and academia. Most of the interest has focused on developing assays suitable for high throughput screening (HTS) purposes, but lately an increasing need for validation of these test systems has emerged (Rodrigues and Lin, 2001, Yan and Caldwell, 2001, Kremers, 2002, Coecke *et al.,* 2006). Table 2.2 compares the different sources of *in vitro* cytochrome P450 enzymes used in pre-clinical research. Figure 2.3 also shows the stepwise method of evaluating drug interactions.

2.8.1 Humanized *in vivo* models

These are genetically modified mice lines expressing various human CYP isoforms and chimeric mice with humanized liver (Tateno *et al.,* 2004; Gonzalez and Yu, 2006). They are used with the purpose of creating more predictive models for human response.

2.8.2 Human-derived *in vitro* techniques

2.8.2.1 Primary hepatocytes

Hepatocytes contain all the enzymes responsible for both phase I and II metabolism. Thus, they are used for multiple identification of drugs effect on metabolising enzymes, drug transportation studies, and identifying toxic effect of New Chemical Entity (NCE). There are good *in vitro – in vivo* extrapolations (IVIVEs) in the activities of drugs on CYP isoforms, whether it is inhibition or induction (Gomez-Lechon *et al.,* 2004). The use of primary hepatocytes is limited by the availability of liver tissues and suitable preservation techniques. Though, cryopreservation may be applied, the lifespan for the use of a single hepatocyte batch is still quite short (Bjornsson *et al.,* 2003).

Table 2.2: Comparison of *in vitro* sources of enzymes used in pre-clinical research

S/no	Enzyme sources	Availability	Advantages	Disadvantages
1	Liver homogenates[a]	Relatively good. Commercially available	Contains basically hepatic enzymes	Liver architecture lost. Cofactor addition neccessary
2	Microsomes[a]	Relatively good. Transplantation or commercial sources	Contains most important rate limiting enzymes. Relatively inexpensive Easy storage	Contain only phase I enzymes and UGTs. Requires strictly specific substrate or antibodies for individual DME's Cofactor addition necessary
3	cDNA expressed individual CYP enzymes[b]	Commercially available	Can be utilized with HTS substrates. The role of individual CYP's in the metabolism can be easily studied	The effect of only one enzyme at a time can be studied
4	Primary hepatocytes[c, d]	Difficult to obtain Fresh tissue needed Commercially available	Contains the whole component of DME's cellularly integrated. The induction effect of an New chemical Entity (NCE) can be studied. Cryopreservation possible.	Requires specific techniques and well established procedures Limited viability
5	Liver slices[e]	Difficult to obtain. Fresh tissue needed.	Contains the whole complement of DME's and cell-cell connections. The induction effect of an NCE can be studied. Cryopreservation possible.	Requires specific techniques and well established procedures Limited viability
6	Immortalised cell lines[f]	Available at request Only few adequately characterised cell lines exist	Non-limited source of enzymes	The expression of most DME's is poor Genotype/phenotype instability.

[a]Kremers (2002); [b]Rodrigues (1999); [c]Guillouzo (1995); [d]Gomez-Lechon *et al.*, (2004); [e]Ferrero and Brendel (1997).

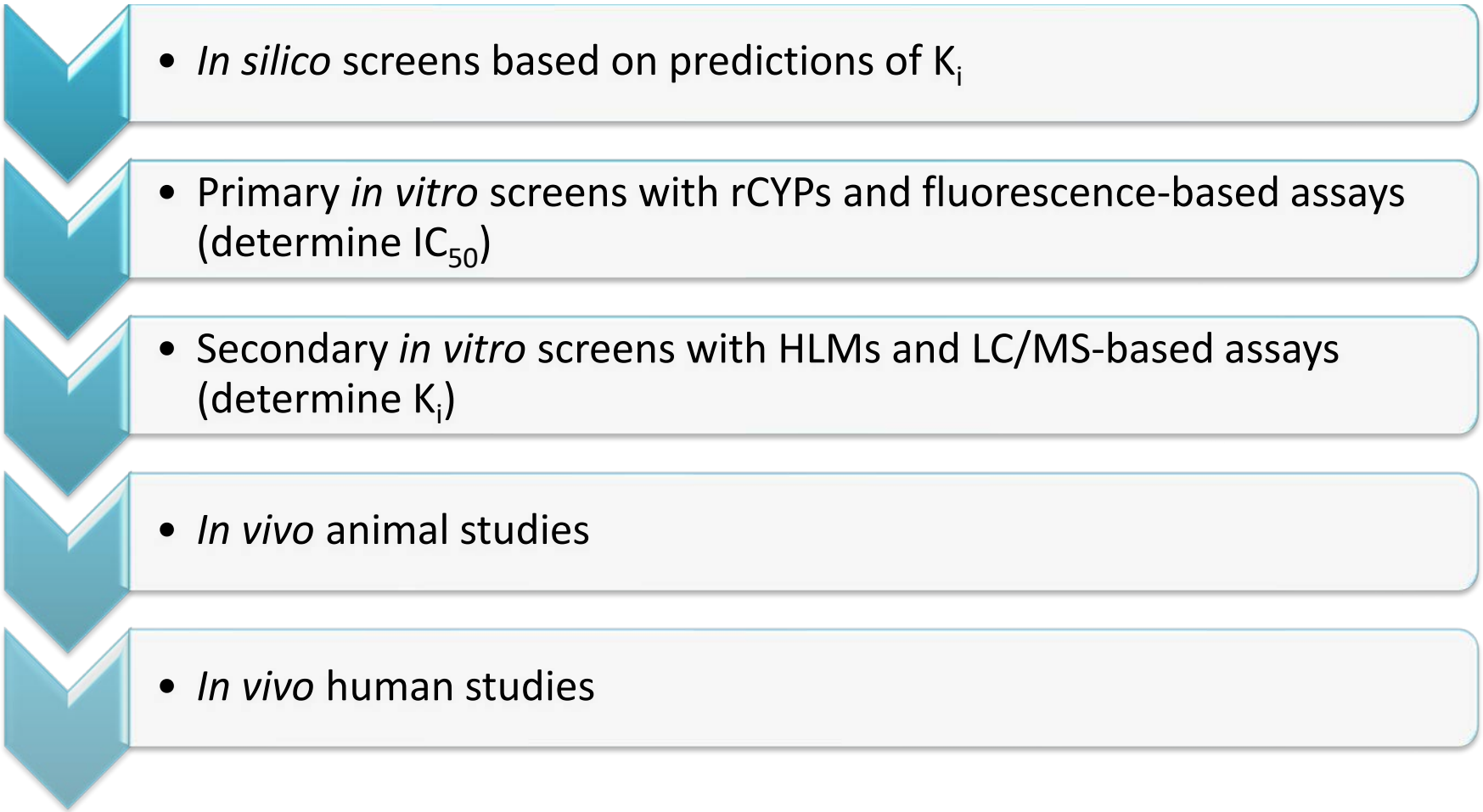


Fig 2.3. Representative steps of a drug-interaction screening process of new chemical entity (NCE) (modified from Rodrigues and Lin, 2001).

2.8.2.2 Immortalised cell lines

Immortalised cell lines are permanent cell lines which usually express one or sometimes more than one CYP isoforms for many years e.g. HepG2, BC2 and A549 which are cell lines derived from the liver and lungs (Hukkanen *et al.,* 2000). Another example of immortalised cell line is the human hepatoma-derived cell line, HepaRG. It contains many drug metabolising enzymes and can be used in drug metabolism and toxicity studies (Aninat *et al.,* 2006).

2.8.2.3 Liver slices

Precision-cut liver slices are used for the study of transport of NCE and for metabolism study since it retains most of the organ functions because it contains most of the metabolising enzymes and the liver transport systems are still intact. Precision-cut liver slices are very useful in the study of induction of NCE; however its maintenance is cumbersome. Cryopreservation is used to extend the life span of the liver slices (Ekins *et al.,* 2000; Edwards *et al.,* 2003). Many studies have shown lower cellular uptake, clearance, and metabolic capacity in liver slices compared to hepatocytes (Ekins *et al.,* 2000), which has probably influenced their popularity.

2.8.2.4 Subcellular fractions

Subcellular fractions are very easy to prepare, reproduce, use, and store. They can be obtained from liver samples as homogenates subcellular fractions, or it can be prepared as microsomes. The homogenate has the full complement of phase I and II enzymes. The microsomal fractions are produced from endoplasmic reticulum after homogenisation and differential ultracentrifugation. Microsomes contain CYPs and UGTs. Both microsomes and liver homogenates are the most widely used *in vitro* system for drug metabolism studies especially CYP-inhibition studies (Pelkonen *et al.,* 2005). Reactivation of loss of enzyme activity during preparation can be achieved by the addition of appropriate cofactors like NADPH, uridine diphosphate glucuronic acid (UDPGA), glutathione (GSH) and 3-phosphoadenosine-5-phosphosulphate (PAPS) (Pelkonen *et al.,* 2005; Coecke *et al.,* 2006). Immediate freezing of the fresh tissue also prolongs its life span.

2.8.2.5 Complementary DNA (cDNA) expressed CYPs

Complementary DNA has been commercially available for several years. Recombinant CYPs are from cDNA in bacterial, yeast, and mammalian cells. They are isolated heterogeneous human CYP enzymes, expressed as single enzymes at a time. They are suitable for high throughput screening (HTS) purposes and can be utilised to ascertain whether an NCE is a substrate for a particular CYP isoform and what metabolite is generated by that specific enzyme (McGinnity and Riley, 2001).

2.8.2.6 Novel cell-based technologies

Future applications for drug metabolism studies include bioartificial liver systems and stem cell-derived cultures and assays (Tsutsui *et al.,* 2006). Preliminary studies concerning the expression, inhibition, and regulation of CYPs in these settings have already been published, making these technologies quite interesting possibilities for future drug metabolism studies.

2.8.3 Computational *in silico* methods

This uses computer-based methods in the prediction of ADME properties of NCEs. *In silico* models provide high throughput and are applied in the very early stages of the drug development process. These approaches can be roughly classified as ligand-based pharmacophore and quantitative structure activity relationship (QSAR), protein-based (crystallographic protein and homology models), and ligand-protein interaction models (ligand-protein docking) (de Groot and Ekins, 2002; de Graaf *et al.,* 2005). These systems are capable of predicting the potential sites of metabolism in the drug molecule and to further provide metabolic trees and pathways with estimations about the likelihood for the production of each metabolite (Langowski and Long, 2002; Button *et al.,* 2003; Coecke *et al.,* 2006).

2.9 Herb-drug interactions (HDIs)

Studies in United Kingdom, Japan and Nigeria have shown that hospitalised patients also take herbal products with their medication in the hospital (Wong *et al.,* 2003; Fakeye *et al.,* 2009; Holst *et al.,* 2009). Among those who take herbal medicines with conventional drugs, 70% fail to inform their health care providers complicating the issue of risk of

herb-drug interactions and consequent adverse drug interactions. Understanding of the mechanisms responsible for herb-drug interactions (HDIs) is important in identifying and preventing HDIs that may lead to ADRs and sometimes the beneficial effects of HDIs could be harnessed for positive therapeutic health outcomes. Most HDIs are as a result of the herb modulating the pharmacokinetic properties of coadministered drug (Shi and Klotz, 2012).

Many patients who use herbs concomitantly with their medications believe that herbs are safe but are unaware of the potential consequences of HDIs. There are reports of mild to serious side effects of HDIs, such as cardiovascular diseases, chest pain, abdominal pain, organ failures and headache (Palmer *et al.*, 2003; Timbo *et al.*, 2006; Shalansky *et al.*, 2007). A study showed that about one-third of patients fail to disclose to their physicians or health-care providers that they are taking herbs (Miller *et al.*, 2008).

2.9.1 Consequences of herb drug interactions

Herbs are sometimes coadministered with conventional drugs, raising the potential of herb-drug interactions. An extensive review of the literature identified reported herb-drug interactions with clinical significance, many of which are from case reports and limited clinical observations (Sorensen 2002; Hu *et al*., 2005; Izzo and Ernst 2009).

Reported cases showed that garlic causes postoperative bleeding and spinal epidural hematoma when used with aspirin or anticoagulants by inhibiting platelet aggregation in patients with coronary artery disease (Rose *et al.*, 1990; Burnham, 1995). Another herb that causes clinically significant herb-drug interactions is St. John's Wort which causes reduction in the level of warfarin, verapamil and statins due to its induction of CYP3A4, CYP2C9, CYP1A2 and P-glycoprotein transporter (Jiang *et al.*, 2004; Mohutsky *et al.*, 2006; Izzo and Ernst, 2009). *Ginkgo biloba* causes spontaneous bleeding when coadministered with aspirin, ibuprofen, or warfarin (Bressler, 2005). Panax ginseng induces mania when used with phenelzine and St. John’s Wort reduces the plasma concentrations of midazolam, digoxin, and indinavir (Hu *et al.*, 2005).

2.10 *Hibiscus sabdariffa*

Hibiscus sabdariffa is known by different names in different parts of the world. It is called “Isapa” or “*Zobo*” in the western part of Nigeria, “*Zoborodo*” in the northern part of Nigeria, “Roselle” or “Rosella” in Australia and Germany, “Bissap” in some other West Africa countries including Senegal, Ghana, Benin and Congo, “Karkade” in Egypt, Saudi Arabia and Sudan, “Sorrel” in the Caribbean and in Latin America while in China it is called “Luo Shen Hua” (Ali *et al.,* 2005; Mahadevan *et al.,* 2009). It is an annual or perennial herb which is native to the tropics. It could grow up to 2 – 2.5 m tall (Fig 2.4 and 2.5). It takes about six months to mature and is popularly used as vegetables, teas and beverages, jams and preservatives. As teas in some part of the world, it is brewed in hot water for 10 - 30 minutes and served, often times flavoured with sugar. In Nigeria it is a popular drink at social gatherings (Kolawole and Maduenyi 2004; Fakeye *et al.,* 2007a)

2.10.1 Phytochemistry of *Hibiscus sabdariffa*

The calyces of *Hibiscus sabdariffa* contain a lot of vitamins and minerals such as iron, thiamine, niacin, and riboflavin among others while the leaves contain calcium and phosphorus as shown in Table 2.3 (Mahadevan *et al.,* 2009). The plant has many useful phytochemicals like gossypetin, sabderitin, hibiscetin e.t.c as shown in Table 2.4 (Mahadevan *et al.,* 2009). .

2.10.2 Pharmacological studies of *Hibiscus sabdariffa*

Hibiscus sabdariffa has been in use for decades as traditional herbal remedy for many diseases some of which are outlined below:

2.10.2.1 Antihypertensive

Antihypertensive properties of the extracts of *Hibiscus sabdariffa* has been demonstrated in both animal and human studies. The aqueous extract of the calyces of *H. sabdariffa* is cardioprotective in rats (Odigie *et al.,* 2003) and significantly reduced systolic and diastolic blood pressure in hypertensive rats (Onyenekwe *et al.,* 1999), similarly two independent studies reported 11.2% and 10% reduction in blood pressure in patients with stage I hypertension (Faraji and Tarkhani, 1999; Herrera-Arellano *et al.,* 2004).

Fig 2.4: ***Hibiscus sabdariffa*** **plant.**

(http://davesgarden.com/guides/pf/showimage/296747/)

Fig 2.5 Calyces of *Hibiscus sabdariffa* plant.

(https://wormandflowers.wordpress.com/tag/gongura-plant/)

Table 2.3: Physicochemical constituents of the fresh calyces and leaves of *H. sabdariffa*

Constituents	Calyces (fresh)*	Leaves (fresh)
Moisture	9.2g	86.2%
Protein	1.145g	1.7-3.2%
Fat	2.61g	1.1%
Fibre	12.0g	10%
Ash	6.90g	1%
Calcium	12.63mg	0.18%
Phosphorus	273.2mg	0.04%
Iron	8.98mg	0.0054%
Carotene	0.029mg	——
Thiamine	0.117mg	——
Riboflavin	0.277mg	——
Niacin	3.765mg	——
Ascorbic Acid	6.7mg	——

*g or mg/100g. Adapted from Mahadevan *et al.*, 2009.

Table 2.4: Phytochemicals of *H. sabdariffa*

Part of the plant	Chemical constituents
Flower	Carbohyrates, arabinans, mannose, sucrose, thiamin, xylose, mucilage, niacin, pectin, proteins. Fat, arabinogalactans, rhamnogalacturans, riboflavin, β-carotene, phytosterols, citric acid, ascorbic acid, fruit acids, maleic acid, malic acid, hibiscic acid, oxalic acid, tartaric acid, (+)-allooxycitronic acid-lactone,allohydroxycitric-acid, glycoside acid, utalonic acid, protocatechuic acid, cyanidin-3-glucoside, cyanidin-3-sambubioside, cyanidin-3-xyloglucoside, delphinidin-3-xyloglucoside, delphinine, gossypetin, gossypetin-3-glucoside, hibiscetin, hibiscin, hibiscitrin, sabdaretin, sabdaritrin, fibre (crude), resin, fibre (dietery), mineral and ash.
Seed	Starch, cholesterol, cellulose, carbohydrates, campesterol, β-sitosterol, ergosterol, propinonic acid, pentosans, pelargonic acid, palmitoleic acid, palmitic acid, oleic acid, myristic acid, methanol, malvalic acid, linoleic acid, sterculic acid, caprylic acid, formic acid, cis-12,13-epoxy-cis-9-octadecenoic acid, isopropyl alcohol, isoamyl alcohol, ethanol, 3-methyl-1-butanol, fibre and minerals.
Leaf	A-Terpinyl acetate, anisaldehyde, β-sitosterol, β-D- galactoside, β-sitosteryl benzoate, niacin, fat, isoamyl alcohol, iso-propyl alcohol, methanol, 3 methyl-1-butanol, benzyl alcohol, ethanol, malic acid, fibre and ash.
Fruit	A-Terpinyl acetate, pectin, anisaldehyde, ascorbic acid, calcium oxalate, caprylic acid, citric acid, acetic acid ethanol, formic acid, pelargonic acid, propionic acid, isopropyl alcohol methanol, benzyl alcohol, 3-methyl-1-butanol, benzaldehyde and minerals
Root	Tartaric acid and saponin

Adapted from Mahadevan *et al.,* 2009

A double blind reference controlled trial reported a significant reduction in blood pressure in groups of patients who took *H. sabdariffa* compared to the captopril group (Herrera-Arellano *et al.,* 2004).

2.10.2.2 Hepatoprotective

The ability of the extract of *H. sabdariffa* to protect the liver against tert-butyl hydroperoxide (t-BHP) induced cytotoxicity and genotoxicity was reported by Tseng *et al.,* (1996) who later demonstrated that the extract can also protect against oxidative stress in liver cells (Tseng *et al.,* 1997). Another study showed the protective effect of the extract of *H. sabdariffa* calyces against cadmium-induced lipid peroxidation of the liver, testes and prostate glands (Asagba *et al.,* 2007) and paracetamol-induced hepatotoxicity (Ali *et al.,* 2003).

The lipid peroxidation was attributed to the anthocyanin content of the plant which has free radical-scavenging properties (Essa *et al.,* 2006), however, despite the protective effect of *Hibiscus sabdariffa* on the liver, prolonged intake of its methano-aqueous extract at 15 doses of 250 mg/kg has been reported to cause liver damage in experimental animals (Akindahunsi and Olaleye, 2003).

2.10.2.3 Antihyperlipidemic

The extracts of *Hibiscus sabdariffa* decreases the level of low density lipoprotein cholesterol, total cholesterol, triglyceride, high density lipoprotein cholesterol and atherogenic index in fructose and, high fat diet and cholesterol fed animals (Carvajal-Zarrabal *et al.,* 2005; Hirunpanich *et al.,* 2006).

2.10.2.4 Antioxidant activity

The antioxidant property of *H. sabdariffa* as reported by Farombi and Fakoya (2005) showed that ethanolic and ethyl acetate fractions of the herb scavenge hydrogen peroxide and inhibits the effect of superoxide anion radicals.

2.10.2.5 Anthocyanin content

The anthocyanins present in *Hibiscus sabdariffa* are cyanidin-3-glucoside, delphinidin-3-glucoside, delphinidin-sambubioside, and cyanidin-3-sambubioside. These have been proposed to be responsible for the numerous antioxidant properties of the plant (Aurelio *et*

al., 2008). Apart from the antioxidant activity of the anthocyanin content of *H. sabdariffa,* it has anticarcinogenic activity (Tsai *et al.,* 2002). The anthocyanins gives the bright red colour to the calyces of *H. sabdariffa*. The stability of the anthocyanin depends on the structure of anthocyanins present in the plant, pH, temperature, oxygen, light, and water activity. Others factors include enzymatic degradation and interactions of the anthocyanin with food components (Jackman and Smith, 1996). The activity of the anthocyanins decreases as the pH of the medium increases (Sukhapat *et al.,* 2004).

2.10.2.6 Anticancer

The anthocyanin content of *Hibiscus sabdariffa* causes apoptosis of cancerous cells in HL-60 cells and RAW2647 cells. *Hibiscus sabdariffa* phytochemicals were also reported to inhibit low density lipoprotein (LDL) oxidation and oxidized low density lipoprotein (oxLDL) mediated macrophages apoptosis which makes it a chemoprotective agent (Chang *et al*., 2005; Chang *et al*., 2006). The protocatechuic content of *Hibiscus sabdariffa* has also been demonstrated to possess antitumor properties in animal models (Tseng *et al.,* 1998).

2.10.3 Pharmacokinetic studies of *H. sabdariffa*

The aqueous extract of *Hibiscus sabdariffa* has been shown to cause reduction in the elimination of acetaminophen and diclofenac, and reduction in the bioavailability of chloroquine (Mahmoud *et al*., 1994; Kolawole and Maduenyi, 2004; Fakeye *et al*., 2007a). The mechanism responsible for this pharmacokinetic herb-drug interaction has not been fully elucidated. A study in 2006 showed that the aqueous extract of *H. sabdariffa* had no significant effect on total CYP contents and the activities of CYP 1A1, 1A2, 2B1, 2B2, 2E1 and 3A4 in rats (Prommetta *et al.,* 2006).

2.11 Applying *in vitro* results to the *in vivo* situation

The *in vitro* - *in vivo* extrapolation is used to predict metabolic clearance and drug interactions. The process of using *in vitro* models to predict *in vivo* drug interactions are still in its infancy, and extensive validation of this approach is needed (Chen *et al*., 2012). *In vitro* models predictive of *in vivo* drug interactions will be essential for rapid, cost-effective screening of pharmaceutical compounds and would be important for reducing risks to patient. Currently, these models are constructed from a combination of laboratory

and theoretical components. Ideally, in a valid model, the *in vivo* decrease in clearance caused by coadministration of an inhibitor would be specifically predicted by the decrease in reaction velocity (e.g., formation rate of a metabolite) for the same compound *in vitro* when the inhibitor is present in the same concentration (Chen *et al*., 2012).

However, presently available models contain a number of weaknesses and assumptions that make scaling of *in vitro* data to the clinical situation complicated and not always accurate (Chen *et al*., 2012). Poor predictions occur with compounds that have flow-dependent hepatic clearance, with mechanism-based inhibition, and with compounds that concurrently induce and inhibit enzyme activity. In addition, inhibitor and substrate plasma concentrations are not always proportional to the inhibitor and substrate concentrations to which the enzyme is exposed (Chen *et al*., 2012).

To establish the feasibility of *in vitro* to *in vivo* scaling, most currently reported predictions of inhibitory drug interactions are retrospective. Presently available methods allow a general assessment of what may occur i.e., an unlikely interaction versus a probable interaction (Thummel and Wilkinson, 1998; Wienkers and Heath, 2005). However, to be most useful, *in vitro* data should not only indicate the possibility of an interaction but also predict its magnitude and clinical importance. Until such a time, the clinical study remains the ultimate means by which a drug interaction and its importance can be assessed.

2.12 Prediction of drug interactions

Obach *et al.*, (2006) reported that the *in vitro* - *in vivo* extrapolation is reliable for the prediction of drug interactions for *in vivo* from *in vitro* data for CYP1A2, CYP2C9, CYP2C19, and CYP2D6, but for CYP3A4 effects of the intestinal and hepatic CYP3A4 should be considered in extrapolating the data.

2.13 Evaluation of herb-drug interactions in preclinical animal models.

Animal models are key experimental protocols in the development of drugs. Though, important deduction can be made from *in vitro* data, important parameters relating to the disposition of the drug or xenobiotics are only determinable from *in vivo* data (Kremers 2002). These important parameters include the total clearance of the xenobiotics from the

body and the contribution of the metabolic and excretory route to this clearance (Bertz and Granneman 1997; Chen *et al*., 2012). Animal models can also be used to determine exposure to metabolites after drug administration. Generally, *in vitro* data can be extrapolated to know if there is need for drug interaction studies in human (Bjornsson *et al*., 2003; Chen *et al*., 2012). In some cases, it is possible to use appropriate experimental animal models to gain insight into drug-drug interactions in human for cases where such experiments cannot be performed in human (Bertz and Granneman 1997; Bjornsson *et al*., 2003; Chen *et al*., 2012). Despite the importance of animal model studies, a major flaw is the differences between the metabolic and transport pathways in animals and humans since animals can have enzymes and transport systems orthologs that are quite different from humans (Martignoni *et al*., 2006; Chu *et al*., 2013).. These differerences in tissue expression or substrate specificity may make the extrapolation of animal studies to human cumbersome (Martignoni *et al*., 2006; Chu *et al*., 2013).

2.14 Purpose of herb-drug interaction studies and protocol for human studies

The main purpose of herb-drug interaction study in human is to determine the component of the herb responsible for the herb-drug interactions and sometimes the possible mechanisms of these interactions (Food and Drug Administration, 2012).. In addition, herb-drug interaction studies may pin-point potentially dangerous interactions and beneficial interactions (Food and Drug Administration, 2012)..

Herb-drug interactions study in human follows the pattern of food-drug interaction study guideline as reviewed previously by Gurley (2012) and Won *et al* (2012) and is similar to the evaluation of drug-drug interactions as outlined by the US Food and Drugs Agency guidelines (Food and Drug Administration, 2012).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Ethical consideration

The study protocol was approved by the University of Ibadan/University College Hospital Ethical Review Committee with approval numbers UI/EC/11/0073 and UI/EC/12/0150.

3.2 Study design

A cross-sectional study design was used for the preliminary survey on the use of aqueous beverage of *Hibiscus sabdariffa* calyces. An N-in-one design (Tolonen *et al.*, 2007) was used to evaluate the *in vitro* inhibitory potentials of the extracts of calyces of *Hibiscus sabdariffa* on selected cytochrome P450 (CYP) isoforms.

In determining the effect of aqueous beverage of *Hibiscus sabdariffa* on phenotypic ratios of caffeine and metoprolol for CYP1A2 and CYP2D6 isoforms respectively, and the pharmacokinetic parameters of caffeine and simvastatin; randomized two-period cross-over designs were employed.

Factorial experimental designs were used to investigate the interaction between aqueous extract of *Hibiscus sabdariffa* and simvastatin.

3.3 Study site

The study was carried out in the Halls of residence and offices in the University of Ibadan and the research laboratory of Department of Clinical Pharmacy and Pharmacy Administration. Analyses of biological samples were done in the Department of Clinical Pharmacy and Pharmacy Administration, the Multidisciplinary Central Laboratory University of Ibadan, the Central Science Research Laboratory Obafemi Awolowo University and African Institute of Biomedical Science and Technology Zimbabwe.

3.4 Survey of use of aqueous beverage of *Hibiscus sabdariffa*

3.4.1 Study setting

The survey of the students was conducted in the Halls of residence in University of Ibadan, while the survey of staff was done in their offices. At the time of the study, the University of Ibadan had twelve halls of residence for both undergraduate and post graduate students and fourteen faculties.

3.4.2 Study period

The study was carried out between August and November, 2012.

3.4.3 Study population

The study was conducted among the staff and students of the University of Ibadan. The student population included the undergraduates and postgraduate students while the staff included teaching and non-teaching members of staff.

3.4.4 Sample size

The total number of teaching staff, non–teaching staff and students in the institution in 2011/2012 academic session were 1364, 1334, and 13,823, respectively. The sample size required was determined using the formula reported by Krejcie and Morgan, (1970).

$$S = \frac{X^2 NP(1-P)}{d^2(N-1) + X^2 P(1-P)} \qquad \text{Equation 3.1}$$

Where

S = required sample size

N = population size

X^2 = the table value of chi-square for one degree of freedom at the desired confidence level (1.96 for 1 degree of freedom)

P = population proportion (assumed to be 0.5 since this will provide the maximum sample size)

d = degree of accuracy expressed as a proportion (0.5)

Sample size for teaching staff:

$$S = \frac{1.96^2 \times 1364 \times 0.5(1-0.5)}{0.05^2 \times (1364-1) + 1.96^2 \times 0.5(1-0.5)} = 300$$

Sample size for non-teaching staff:

$$S = \frac{1.96^2 \times 1334 \times 0.5(1-0.5)}{0.05^2 \times (1334-1) + 1.96^2 \times 0.5(1-0.5)} = 299$$

Sample size for students:

$$S = \frac{1.96^2 \times 13823 \times 0.5(1-0.5)}{0.05^2 \times (13823-1) + 1.96^2 \times 0.5(1-0.5)} = 374$$

Allowing for 10% attrition, the targeted sample size is 973 + 98

The total sample size = 1071.

3.4.5 Inclusion criteria

Every bonafide students of the rooms visited in the halls of residence and members of teaching and non-teaching staff as listed by the departmental secretaries in the selected departments who consented to take part in the study were included.

3.4.6 Exclusion criteria

Students who were not in their allocated rooms, or who did not belong to the rooms visited at the time of the distribution of questionnaire were not included in the study to avoid duplication. Friends and visitors of the legal occupants of each room were also excluded from the study. Traders and other members of the University community who were not students, teaching or non-teaching staff or those visually impaired were excluded from the study.

3.4.7 Research instrument and data collection procedure

3.4.7.1 Study instrument

A structured questionnaire was used in the survey. The instrument was pretested among twenty one members of staff and students (11 students, 6 non-teaching staff, and 4 teaching staff) randomly selected in the Faculty of Pharmacy and the Department of Biochemistry. These participants were not included in the final survey. Based on the outcome of the pretest and the comments of two lecturers from the department of Clinical

Pharmacy, modifications were made in the questionnaire for face and content validity. More options such as the frequency of consumption of aqueous beverage of *Hibiscus sabdariffa,* why participant took the beverage, its use with other medications, discomforts resulting from its use and a list of diseases or ailments the beverage is used to treat or manage were all added to meet the objectives of the study. The final questionnaire had eighteen (18) questions (Appendix A). Chronbach alpha for the scales in the questionnaire was between 0.76 and 0.82.

The questionnaire contained sections on demographic data like age, sex, religion, level of study, status in the university (whether staff or student), previous academic qualifications etc. Information on the pattern and extent of use of water beverage of *Hibiscus sabdariffa*, indications, side effects experienced, and drugs usually coadministered with the beverage were also obtained.

3.4.7.2 Sampling method and procedure

Convenient sampling method was used in administering the questionnaire in the halls of residence. The twelve halls of residence had an average of 56 rooms per hall and each room was usually occupied by students of unrelated disciplines. In the ten undergraduate halls, four students occupy a room while the two postgraduate halls had two students per room. Distribution of questionnaire was done from one room to another and from one hall of residence to another. The occupants of each room were approached and individual consent to participate in the study obtained after detailed explanation of the study. Only consented students were given the questionnaire to fill. In cases where the occupants of the room declined consent or where the room was locked, the next available room in the hall was visited. The last room visited for the day in a particular hall was marked for continuity the next day. Questionnaires were retrieved from the students on the same day of distribution.

The non-teaching staff such as security staff, registry staff and postgraduate school staff, were approached at their duty post. Questionnaires were given to consented participants and retrieved the next day. For teaching staff, the questionnaires were distributed through the secretaries in the randomly selected departments in each faculty. Departments surveyed were randomly selected by using table of random numbers to select one

department from each of the fourteen faculties. In faculties with one academic program like faculty of Pharmacy, all consented staff were selected. Retrieval of questionnaires was also done on the following day through the secretaries.

3.5 Preparation of plant material for *in vitro* and *in vivo* studies

3.5.1 Collection and preparation of plant material

Dried calyces of *Hibiscus sabdariffa* was purchased from Gbagi Market, Ibadan, Oyo State. Samples were identified and authenticated at the Forestry Research Institute of Nigeria (FRIN). Herbarium specimen number FHI 106934 was allocated. It was dried in the oven, at 40^{o}C until a constant weight was obtained and pulverized with blender (Kenwood®, Model Number: OWBL436003, China) for three minutes.

3.5.2 Phytochemical analysis of powdered *Hibiscus sabdariffa* calyces

3.5.2.1 Test for alkaloid

One gram powdered sample was extracted with 10 mL of 10% hydrochloric acid on a water bath for about 10 minutes. The extract was filtered and the pH of the filtrate was adjusted to 6 - 7 using ammonia solution. The filtrate was divided into three parts in different test tubes and drops of Dragendoff, Meyer and Wagner reagents were added respectively to the content of each test tube. Formation of precipitate with the Dragendoff reagent confirmed the presence of alkaloid in the powdered sample (Evans, 2009).

3.5.2.2 Test for cardiac glycosides

About 1 g of the powdered sample was extracted with 10 mL of 80% v/v alcohol on a water bath for about 5 minutes. It was filtered while hot and allowed to cool to room temperature. The filtrate was diluted with distilled water, mixed thoroughly and few drops of lead acetate were added in a test tube. This was allowed to stand for few minutes and filtered afterwards. The resultant filtrate was partitioned into three volumes of chloroform and the combined chloroform extracts were divided into two test tubes and content evaporated to dryness on a water bath and the following tests were performed (Evans, 2009):

3.5.2.2.1 Kedde test

One portion of the dried residue above was mixed with 3, 5- dinitro benzoic acid in ethanol (2%). The solution was made alkaline with 5% sodium hydroxide after mixing. A brownish-purple colour confirmed the presence of cardiac glycosides (Evans, 2009).

3.5.2.2.2 Keller Killiani test

The other portion in section 3.5.2.2 above was dissolved in 3 mL ferric chloride reagent. Two milliliter of concentrated sulphuric acid was poured down the side of the test tube carefully in order to form a layer below the acetic acid. A reddish brown ring at the interphase and green colour in the acetic acid layer confirmed the presence of cardiac glycoside (Evans, 2009).

3.5.2.3 Test for anthraquinone

One gram of powdered sample was boiled in 2 mL of 10% hydrochloric acid for 5 minutes, filtered while hot and allowed to cool to room temperature. The cooled filtrate was partitioned against equal volume of chloroform and the chloroform layer transferred to a clean test tube using clean pipette. Equal volume of 10% ammonia solution was added to the content of the test tube. A rose red pink colour indicated the presence of anthraquinones (Evans, 2009).

3.5.2.4 Test for saponins and tannins

Approximately 1 g of powdered sample was boiled with distilled water and filtered while hot, and allowed to cool to room temperature. About 2.5 mL of the filtrate was transferred into a test tube, diluted with equal volume of distilled water, and properly mixed. The solution was divided into two equal parts. Tests for the presence of saponins and tannins were carried out on the solution (Evans, 2009).

3.5.2.4.1 Saponins

One part of the solution in 3.5.2.4 above was shaken vigorously for about two minutes and the formation of foam that lasted for a few minutes confirmed the presence of saponins (Evans, 2009).

3.5.2.4.2 Tannins

To the second solution in 3.5.2.4 above was added drop of 0.1% of ferric chloride solution. A blue black coloration and precipitate confirmed the presence of tannins in the sample (Evans, 2009).

3.5.2.5 Test for flavonoids

One gram of the sample was boiled in 50 mL of 80% v/v alcohol, filtered while hot, and concentrated on water bath. Three chips of magnesium turnings were dissolved in 2 mL of the filtrate in a test tube. Two milliliter of concentrated sulphuric acid was carefully poured down the side of the test tube and a purple or red colour confirmed the presence of flavonoids (Evans, 2009).

3.5.3 Preparation of extracts for *in vitro* and *in vivo* studies

3.5.3.1 Ethanolic extract

Dried powdered calyces (100 g) of the plant were infused with 1 L of absolute ethanol at room temperature for 4 hours. The solution was decanted and the residue further extracted with another 1 L of absolute ethanol for 4 hours. The filtered solutions were pooled, sieved with a stainless steel sieve filter of 1 µm pore size, concentrated with rotary evaporator (Buchi Rotavapor R-210, model number: 0800014803, Switzerland) at 40^{o}C and freeze dried (Lyotrap Plus® Freeze Drier, Model Number: 912350, Great Britain). The freeze dried extract was stored at 4^{o}C until required.

3.5.3.2 Aqueous extract

Powdered dried calyces of *Hibiscus sabdariffa* (500 g) was extracted according to the method described by Fakeye (2008). Briefly, 500 g of the powdered dried calyces of *Hibiscus sabdariffa* was macerated in 1.5 L of boiled distilled water for four hours and filtered. The residue was further macerated in another 1 L of boiled distilled water for another 4 hours. The mixture was filtered. The filtrates were pooled, concentrated with rotary evaporator at 40^{o}C and freeze dried. The freeze dried aqueous extract was stored at 4^{o}C until needed for experiment.

3.5.3.3 Preparation of aqueous beverage of *Hibiscus sabdariffa*

Aqueous beverage of *Hibiscus sabdariffa* was prepared using the method described by Fakeye *et al.,* (2007a). Briefly, 300 g of powdered dried calyces of *Hibiscus sabdariffa* was infused in 11 L of previously boiled distilled water for four hours. The mixture was filtered and made up to 11 L. The filtrate is referred to as aqueous beverage of *Hibiscus sabdariffa* (ABHS). Three hundred milliliter (300 mL) of the beverage was found to be equivalent to 8.18 g of dried calyces of *Hibiscus sabdariffa.*

The pH of the ethanolic and aqueous extracts of Hibiscus sabdariffa including the aqueous beverage was determined.

3.5.4 Anthocyanin determination

The total monomeric anthocyanin (TMA) content of the extracts of *Hibiscus sabdariffa* was determined using Cyanidin-3-glucoside using the pH differential method described by Wrolstad *et al* (2005). Briefly, an aliquot of each extract of *Hibiscus sabdariffa* was taken before concentrating the extracts. Appropriate dilution factor was determined by diluting the test portion of the extracts with pH 1.0 buffer (potassium chloride, 0.025 M) until absorbance at 520 nm was within the linear range of the spectrometer used (PerkinElmer Lambda 25UV/Vis Spectrophotometer, Model Number: 501508080511, Singapore). The dilution factor obtained was used to prepare two dilutions of the extract solution, one with pH 1.0 buffer and the other with pH 4.5 buffer (sodium acetate, 0.4 M). This was done in triplicate. The absorbance of these two dilutions were determined at 520 nm and 700 nm and read against a blank cell filled with distilled water. The difference in this absorbance was used in calculating the TMA present in 100 g of the powdered plant material.

The anthocyanin content of the extracts was determined using the formula

$$TMA = \frac{A \times MW \times DF \times 10^3}{\in \times I} \qquad \text{Equation 3.2}$$

Where

$$A = (A_{520nm} - A_{700nm})pH\ 1.0 - (A_{520nm} - A_{700nm})pH\ 4.5 \qquad \text{Equation 3.3}$$

MW = molecular weight in g/mol of cyaniding-3-glycoside (449.2 g/mol);

ε = molar extinction coefficient in L/mol/cm (26 900 L/cm/mol for Cyanidin-3-glucoside)
DF = dilution factor; I = path length in cm.

3.6 *In vitro* evaluation of inhibitory potential of *Hibiscus sabdariffa* extracts on eight cytochrome P450 isoforms

3.6.1 CYP-inhibition experiments

Pooled microsomes for metabolite profiling and CYP-inhibition studies were obtained from BD Biosciences Discovery Labware (Bedford, MA). The human liver microsomes (HLM) pool containing 20 mg protein/mL (Lot# 99268) consisted of liver samples from 25 donors of both genders. The cocktail-approach for elucidating inhibition towards CYP-specific model reactions had been described in detail in previous works (Turpeinen *et al*., 2005; Tolonen *et al*., 2007). Briefly, each incubation mixture contained 0.5 mg microsomal protein/mL, 0.1 M phosphate buffer (pH 7.4), 1 mM nicotinamide adenine dinucleotide phosphate (NADPH), and nine probe substrates for major drug-metabolizing CYPs. The substrates used, their target CYP isoforms, and final concentrations of the substrates in the incubation mixtures are shown in Table 3.1.

The dried ethanolic extract was dissolved in methanol and added to the incubation mixtures to obtain final concentrations of 0.001, 0.01, 0.1, 1, 10, 100, and 1000 µg/mL. The final amount of methanol in the incubation mixtures was 1% (v/v). The reaction mixture, in a final volume of 200 µl, was preincubated for 2 minutes at 37°C in a shaking incubator block (Eppendorf Thermomixer 5436, Hamburg, Germany) before the reaction was initiated by the addition of NADPH. Each reaction was terminated after 20 minutes by adding 100 µL of ice-cold acetonitrile containing phenacetin (1 µM) as an internal standard (IS) for the extracts of *Hibiscus sabdariffa*. Samples were subsequently cooled in an ice bath to precipitate the proteins and stored at -20°C until analysed. Duplicate incubations were carried out.

For aqueous extract of *Hibiscus sabdariffa* a cocktail of nine CYP-specific substrates, buffer, and human liver microsomes were preincubated for 6 minutes, with and without the extract.

Table 3.1: The conditions and probes substrates used for *in vitro* evaluation of ethanolic extract of *Hibiscus sabdariffa* for selected cytochrome P450 isoforms.

Enzyme	Substrate	Concentration	Metabolite
CYP1A2	Melatonin	5µM	6-OH-melatonin (OH-MEL)
CYP2A6	Coumarin	2µM	7-OH-coumarin (OH-COU)
CYP2B6	Bupropion	2µM	OH-bupropion (OH-BUP)
CYP2C8	Amodiaquine	5µM	Desethylamodiaquine (desEt-AMO)
CYP2C9	Tolbutamide	8µM	OH-tolbutamide (OH-TOL)
CYP2C19	Omeprazole	5µM	5-OH-omeprazole (5OH-OME)
CYP2C19	Omeprazole	5µM	Desmethylomeprazole (deM-OME)
CYP2D6	Dextromethorphan	1µM	O-desmethyldextromethorphan (O-deM-DEX)
CYP3A4	Midazolam	1µM	1-OH-midazolam (1OH-MID)
CYP3A4	Testosterone	5µM	6β-OH-testosterone (6β-OH-TES)
CYP3A4	Omeprazole	5µM	Omeprazole sulphone (SO2-OME)
CYP3A4	Omeprazole	5µM	3-OH-omeprazole (3OH-OME)

Enzymatic reactions were started by adding NADPH and terminated after 15 minutes by adding 100 μL of ice-cold acetonitrile. Samples were centrifuged and supernatants were collected for analyses.

The samples were analysed using liquid chromatography tandem mass spectrometer (LC/MS-MS) to determine the level of metabolites in the absence and presence of the extract. Standard positive controls were also used for both extracts and quantification based on relative peak areas were used. The probe reactions used for the aqueous extract are listed in Table 3.2. To evaluate the possible matrix effects to LC/MS/MS, analyses of probe metabolites by the complex plant extracts; extra control incubations where the extracts were spiked after termination of metabolic reactions were performed.

3.6.2 Liquid chromatography-mass spectrometry conditions

The analysis of probe metabolites from CYP-specific marker reactions were conducted with a LC/MS/MS method modified from an earlier study (Tolonen *et al.,* 2007). Briefly, a Waters Alliance 2695 HPLC system (Waters Corp., Milford, MA) was used together with a Phenomenex Kinetex C18 column (2.1 mm × 50 mm column with 2.6 μm particle size) and an on-line filter at 40°C. The injection volume was 6 μL. The HPLC eluents were aqueous 0.1% acetic acid (pH 3.2) as mobile phase A and methanol as mobile phase B. The gradient elution from 98%A + 2%B to 50%A + 50%B to 20%A + 80% B was applied in 0 to 0.5 to 3.0 min, followed by column equilibration, giving a total time of 7 min/injection.

The eluent flow rate was 0.4 mL/min. The data was acquired using a Waters Quattro Ultima triple quadrupole mass spectrometer equipped with a Z-spray ionization source. The Multiple Reactions Monitoring (MRM) mode used both positive and negative polarity (separate injections for each polarity). For all compounds, the capillary voltage used was 4200 V in positive ion mode and 3500 V in negative ion mode. The desolvation and source temperature were 400^{o}C and 150^{o}C, respectively. Nitrogen was used both as the drying and nebulizer gas. The collision cell argon pressure was set to 3.5×10^{-3} mbar. The MRM transitions and the compound dependent parameters are displayed in Table 3.3. The instruments were controlled using MassLynx 4.1 software.

.**Table 3.2: The conditions and probes used for *in vitro* evaluation of aqueous extract of *Hibiscus sabdariffa* for selected cytochrome P450 isoforms.**

Enzyme	Substrate	Concentration in the incubation [µM]	Metabolite
CYP1A2	Phenacetin	10	Acetaminophen (ACET)
CYP2A6	Coumarin	2	7-OH-coumarin (7-OH-COU)
CYP2B6	Bupropion	2	OH-bupropion (OH-BUP)
CYP2C8	Repaglinide	5	OH-repaglinide (OH-REPA)
CYP2C9	Diclofenac	5	4'-OH-diclofenac (OH-DICL)
CYP2C19	Omeprazole	5	5-OH-omeprazole (5-OH-OME)
CYP2C19	Omeprazole	5	Desmethylomeprazole (deM-OME)
CYP2D6	Dextromethorphan	1	Dextrorphan (O-deM-DEX)
CYP3A4	Omeprazole	5	3-OH-omeprazole (3-OH-OME)
CYP3A4	Omeprazole	5	Omeprazole sulphone (SO2-OME)
CYP3A4	Testosterone	5	6β-OH-testosterone (6b-OH-TES)
CYP3A4	Midazolam	1	1'-OH-midazolam (1-OH-MDZ)

Table 3.3: Conditions for detecting each CYP probe substrate metabolite(s)

Compound	Multiple Reaction Monitoring mode transition	Cone Voltage (V)	Collision energy (eV)	Polarity	Retention time (min)
Desethylamodiaquine	m/z 328 > 283	28	16	ESI+	2.7 min
Hydroxymelatonin	m/z 249 > 190	22	14	ESI+	3.1 min
Dextrorphan	m/z 258 > 199	42	26	ESI+	3.1 min
Hydroxycoumarin	m/z 163 > 107	32	20	ESI+	3.3 min
Hydroxybupropion	m/z 256 > 238	20	12	ESI+	3.2 min
5-hydroxyomeprazole	m/z 362 > 214	20	12	ESI+	3.6 min
3-hydroxyomeprazole	m/z 362 > 214	20	12	ESI+	3.7 min
Hydroxytolbutamide	m/z 287 > 171	22	17	ESI+	3.5 min
1-Hydroxymidazolam	m/z 342 > 324	35	20	ESI+	4.2 min
Phenacetin (IS)	m/z 180 > 110	32	17	ESI+	3.5 min
Omeprazole sulfone	m/z 362 > 150	32	25	ESI+	4.0 min

MRM – multiple reaction monitoring mode; ESI – electrospray ionization positive (+) or negative (-)

3.7 *In vivo* studies in healthy human volunteers and an animal model

3.7.1 *In vivo* evaluation of inhibitory potentials of the extract of *Hibiscus sabdariffa* on CYP1A2 and CYP2D6 isoforms.

3.7.1.1 Chemicals

High Pressure Liquid Chromatography (HPLC) grade methanol was obtained from Fisher Scientific UK. High performance liquid chromatographic grade formic acid and the following standards: caffeine, paraxanthine, metoprolol, α-hydroxymetoprolol and 7-hydroxywarfarin were obtained from Sigma-Aldrich Germany. High performance liquid chromatographic grade Acetonitrile and acetic acid 96% from Merck KGaA, Germany. Paracetamol powder (99% purity) was obtained from Pharmanova PVT Ltd, Zimbabwe and sodium acetate trihydrate from Amresco. Caffeine 200 mg tablets (Stay Awake®) was purchased from Walgreens Co. Wilmot Rd, USA and Metoprolol 50 mg tablets was purchased from TEVA.

3.7.1.2 Subjects

Twenty seven young healthy volunteers (15 males and 12 females) with age range 17 – 34 years were recruited into the study. The health status of the volunteers was ascertained through a health screening self-administered questionnaire (Appendix B), physical examination and biochemical and hematological screening. All volunteers gave written informed consent (see Appendix C for sample informed consent form). Volunteers were non-smokers and non-pregnant. They were asked to abstain from consuming alcohol, caffeinated beverages, charbroiled foods, grapefruit juice, and any prescription or over-the-counter medication including vitamins and herbal products for at least one week prior to and during each study period.

3.7.1.3 Study conduct

The volunteers were divided into two arms without bias for gender. An open randomized two-periods cross-over design was used (Turpault *et al.*, 2009) in which the first group of volunteers received a cocktail of caffeine 200 mg tablet and metoprolol 50 mg tablet (Turpault *et al*., 2009; Zhu *et al*., 2001). The drugs were self-administered with 300 mL of water in one arm of the study while the other arm of the study group took 300 mL of aqueous beverage of *Hibiscus sabdariffa* prepared as described in section 3.5.3.3 above

with the drug cocktail. A wash-out period of one week was observed for the complete clearance of the drug and aqueous beverage of *Hibiscus sabdariffa* from the body in between experiments.

The administration of the drug cocktail and aqueous beverage of *Hibiscus sabdariffa* was reversed in the second period of the study. All subjects were questioned about any overt adverse effects potentially related to the administered drug during and after each study period. Two volunteers complained of mild stomach ache and one volunteer complained of transient headache. These symptoms which lasted for few hours, were not disabling to the volunteers, and did not require medication. These were part of the volunteers who took the drug cocktail in the first phase of the experiment.

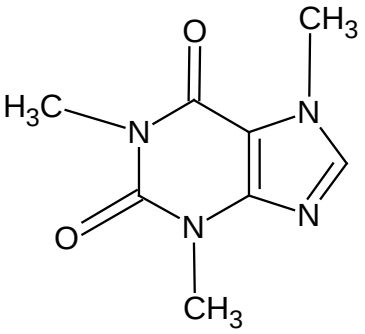
3.7.1.4 Blood sample collection for the determination of metabolic ratio

Blood samples (5 mL) were collected at 4 hours single time point post administration of the drugs (caffeine and metoprolol). The blood samples were centrifuged immediately at 3500 *g* for 10 min; and the plasma transferred into cryotubes and stored at -20^{o}C until analysed.

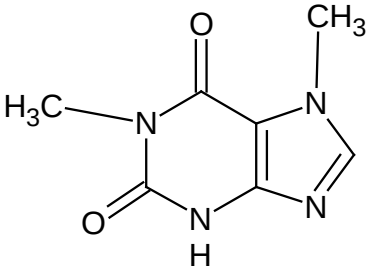
3.7.1.5 Equipment

For the analysis of caffeine and its metabolite, paraxanthine, (Fig 3.1), Hewlett Packard 1100 HPLC equipped with G1311A quaternary pump (serial # DE23921791), a G1379A online degasser, G1330B auto liquid sampler thermostat, a G1329A auto-liquid sampler, G1316A column compartment, and a G1315B diode array detector was used. Data acquisition was achieved with Chemstation software version A.06.03 (509).

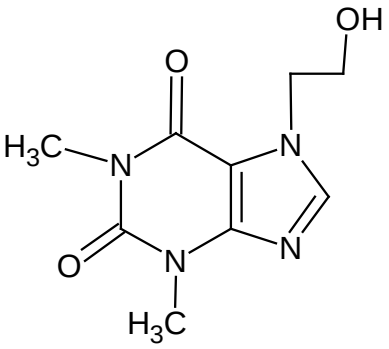
The analysis of metoprolol and α-hydroxymetoprolol (Fig 3.2) was performed with LC-MS/MS using QTRAP 3200 AB SCIEX Mass spectrometer. The instrumentation comprises of a degasser, a variable loop autosampler, a quaternary gradient pump, a reverse phase C18 column, a triple quadrupole mass spectrometer interfaced with a heated electrospray turbo ion source, one roughing vacuum pumps, air compressor, and nitrogen gas generator. The Analyst1.6.2 software was used for optimizing tuning parameters and for data acquisition and processing.



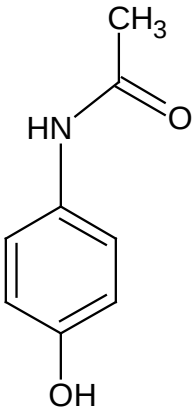
Caffeine



Paraxanthine

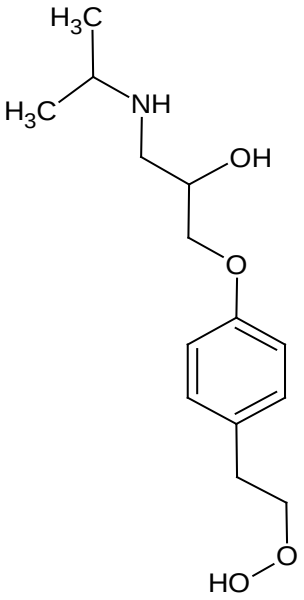


Etofylline

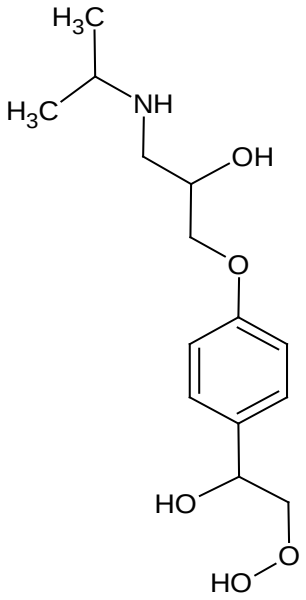


Paracetamol

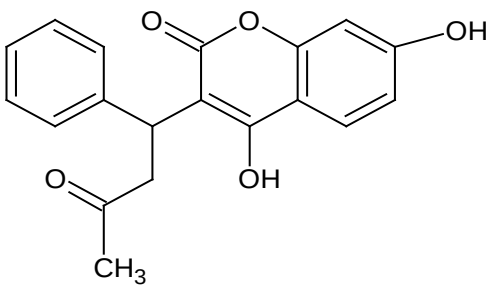
Fig 3.1: Chemical structures of analytes caffeine, paraxanthine, paracetamol (internal standard) and etofylline (internal standard)



Metoprolol



Alpha - hydroxymetoprolol



7 - hydroxywarfarin

Fig 3.2: Chemical structures of metoprolol, α-hydroxy metoprolol and the internal standard 7-hydroxywarfarin

All standard samples were weighed with Mettler Toledo balance (model number AB AT261). pH meter used was Eutech instruments pH 700®. Mobile phase was sonicated with Branson 200 ultra-cleaner (Model 200, Taiwan).

3.7.1.6 Standard solutions

Stock solutions of 1 mg/mL paracetamol (internal standard for the analysis of caffeine and paraxanthine) and 2 mg/mL each of caffeine and paraxanthine were prepared in methanol:distilled water (1:1). Working solutions of caffeine and paraxanthine were prepared from the stock solution by diluting with methanol: distilled water (1:1) to obtain concentrations of 0.25, 1.25, 2.5, 5.0, 25.0, 50.0, 100.0, 150.0, 250.0, and 500.0 µg/mL. The working solution of paracetamol was prepared from the stock solution by diluting with methanol: distilled water (1:1) to obtain concentrations of 12.5 µg/mL. The distilled water used was filtered with Nalgene SFCA 0.2 µm filter.

Stock solutions of 1 mg/ml of metoprolol, α-hydroxymetoprolol and 7-hydroxywarfarin were prepared in 100% acetonitrile. Working standards of 0.25, 1.25, 2.5, 6.25, 12.5, and 25.0 µg/mL for metoprolol and α-hydroxymetoprolol were made from serial dilution of a combined mixture of metoprolol and α-hydroxymetoprolol at 25 µg/ml with acetonitrile: 0.1% formic acid (70:30). Working solution of 500 ng/mL of the internal standard 7-hydroxywarfarin was also made.

3.7.1.7 Sample preparation

For the analysis of caffeine, a simple modification of the method described by Christensen *et al.,* (2003) was used. Briefly, 100 µL of plasma was spiked with 20 µL of the internal standard paracetamol at a concentration of 12.5 µg/mL and 300 µL of ice cold acetonitrile were also added. This was vortexed for 5 min with vortex mixer IKA Genius 3 (model VG 3.S2) and agitated with Eppendoff Thermomixer Comfort (Germany) at 350 *g* and at 4ºC for 10 min. Afterward, it was centrifuged with Eppendoff centrifuge 5415R (Hamburg Germany) at 13,200 *g* and at 4ºC for 10 min. The supernatant was removed into Eppendoff tube and evaporated in a water bath at 45ºC to dryness under a gentle stream of nitrogen with Mayer N-EVAP Analytical Evaporator (Model 112, U.S.A). The residue was reconstituted with 100 µL of mobile phase and 10 µL injected onto the column.

For the sample preparation of metoprolol and its metabolite, 100 µL of plasma was taken into a 1.5 mL Eppendorf tube and 400 µL of ice cold acetonitrile containing 500 ng/mL 7-hydroxywarfarin (internal standard) was added as the extraction solvent followed by vortexing for 30 sec. The mixture was gently agitated for 10 min at 350 *g* at 25^0C. Centrifuged at 13,200 *g* for 10 min at 4^oC and all the clear supernatant was transferred into a 1.5 mL Eppendorf tube. Hundred microliter of clear supernatant was transferred into an HPLC vial for LC/MS/MS analysis.

3.7.1.8 Chromatographic condition

Separation of caffeine and paraxanthine was achieved with a Reverse Phase Hichrom (serial #: KR100-5C18-3968) C18 column (150 mm x 4.6 mm; particle size 5 µm). The mobile phase delivered at a flow rate of 1.0 mL/min consisted of 15% methanol in 25 mM of sodium acetate buffer (3.402 g of sodium acetate trihydrate was dissolved in 1000 mL of distilled water) adjusted with acetic acid to pH 4.0.

Separation of metoprolol and α-hydroxymetoprolol was achieved with a prepacked Luna Phenomenex C18 Analytical Column, Dimensions, 50 mm x 2.0 mm, and 3 µm column. Mass spectrometry was performed using an AB SCIEX 3200 QTRAP mass spectrometer with an electrospray ionization (ESI) source. The ESI was done in the positive Multiple Reaction Monitoring (MRM) mode. The mobile phase consisted of mobile Phase B - HPLC grade water + 0.1% formic acid and mobile Phase C - 100% HPLC grade Acetonitrile used as gradient elution (Table 3.4).

3.7.1.9 Calibration curve

Concentrations of 0.05, 0.25, 0.5, 1, 5, 10, 20, 30, 50, and 100 µg/mL caffeine, and paraxanthine were prepared by spiking 100 µL of blank plasma with 20 µL of each analyte from the respective working solutions. The plasma was also spiked with 20 µL of 12.5 µg/mL of the internal standard, to give final concentration of 2.5 µg/mL. These were prepared for analysis after the separation method described in section 3.7.1.7..

Calibration curves for metoprolol and its metabolite were constructed by spiking 100 µL of plasma with 20 µL of composite mixture of metoprolol and α-hydroxymetoprolol from

Table 3.4 Mobile phase gradient for the analysis of metoprolol and α-hydroxymetoprolol

Step	Time (min)	Flow Rate (µL/min)	B (0.1% formic acid)	C (Acetonitrile)
1	0.00	50	98.00	2.0
2	1.00	50	98.00	2.0
3	1.10	50	98.00	2.0
4	1.20	500	98.00	2.0
6	6.10	500	100.0	0
7	6.70	500	100.0	0
8	6.80	500	98.0	2.0
9	9.00	500	98.0	2.0

the working standards to make calibration standards of 10, 50, 100, 250, 500, and 1000 ng/mL for each analyte.

Calibration curves for caffeine, paraxanthine, metoprolol, and α-hydroxymetoprolol were constructed with Excel by plotting a linear regression curve of the peak area ratio of the analytes to the respective internal standards against concentrations without any weighting factor. This was used to assess the linearity of the calibration curve and to generate the standard calibration equation: y=ax + b i.e. where y is the peak-area ratio, x is the concentration, a, is the slope and, b, is the intercept of the regression line.

3.7.1.10 Method validation for the determination of phenotypic metabolic ratio of caffeine and metoprolol

3.7.1.10.1 Precision and accuracy

For precision and accuracy, known amounts of analytes, caffeine and paraxanthine, with constant amount of the internal standard were added to 100 µL of blank plasma yielding a concentration of 0.05, 0.15, 3.00, and 40.00 µg/mL of paraxanthine; 1, 3, 40 and 80 µg/mL of caffeine and 2.5 µg/mL of paracetamol (IS). These represented the lower limit of quantification (LLOQ), low quality control sample (LQC), medium quality control (MQC), and high quality control sample (HQC). For metoprolol and α-hydroxymetoprolol the LLOQ, LQC, MQC, and HQC were 10, 40, 400 and 800 ng/mL, respectively with 500 ng/mL of 7-hydroxywarfarin as internal standard.

In the determination of intra-day precision and accuracy, six replicates of the quality control samples were analysed three times on the same day while for inter-day precision and accuracy, they were analysed once a day on three different days in a week. Precision was determined as the percentage relative standard deviation (% R.S.D) or percentage coefficient of variation (% C.V) and accuracy was calculated as the percentage of spiked concentration.

3.7.1.10.2. Lower limit of quantification

According to European Medicines Agency, (2014) the lower limit of quantification was determined as the lowest concentration on the calibration curve with acceptable precision (% R.S.D <20%) and accuracy ±20% of theoretical or nominal value.

3.7.1.10.3. Recoveries

Six replicates of LLOQ and the quality control samples LQC, MQC, and HQC were used to determine the percentage recoveries of each analyte (caffeine and paraxanthine; metoprolol and α-hydroxymetoprolol). Known amount of the analytes were added to plain plasma to obtain the quality control concentrations. Neat solutions containing the same concentration of the quality control samples were also analysed and the percentage recoveries was calculated as

$$\%\ Recovery\ = \frac{\text{Peak response ratio of spiked sample with reference to internal standard}}{\text{Peak response ratio of neat solution with reference to internal standard}}\ X\ 100$$

Equation 3.5

3.8 *In vivo* evaluation of the effect of aqueous extract of *Hibiscus sabdariffa* on the pharmacokinetic parameters of caffeine and simvastatin

3.8.1 Reagents

LiChrosolv® methanol and acetonitrile were obtained from Merck KGaA Darmstadt Germany. Lovastatin, simvastatin, caffeine (1. 3, 7, trimethylxanthine) and paraxanthine (1, 7 dimethylxanthine) were purchased from Fluka Sigma-Aldrich, etofyline from Toronto Research Chemicals Incorporation, Toronto Canada. Simvastatin tablets were purchased from TEVA and caffeine tablets (Stay Awake®) from Walgreens Co. Wilmot Rd, USA.

3.8.2 Instrumentation and analytical condition

Agilent HPLC 1200 series equipped with isocratic pump G1310A, online degasser, Rheodyne manual injector fitted with a 20 µL loop, and a UV-detector G1314B was used. Separation of analytes was achieved with Agilent Eclipse XDB column C18 (150 mm x 4.6 mm; 5 µm) and data was acquired with Chemstation software version Rev B.03.02.(341).

3.8.3. Subjects

Six healthy male volunteers with mean age 23.14 ± 3.98 years participated in the study. Their health status was confirmed through hematological and biochemical test and a

health screening questionnaire (Appendix B). Prior to the commencement of the study, all volunteers who gave their written informed consent (Appendix C) were asked to abstain from taking any drug, herbs or herbal medicines, over the counter medications, juice and drinks containing caffeine, charbroiled meat, kolanut and sweets containing coffee or coffee drinks for two weeks before and during the study.

3.8.4 Experimental protocol

The six healthy male volunteers were randomly divided into two arms of the study. The volunteers in the first arm took a cocktail of caffeine 200 mg tablet and simvastatin 40 mg tablet with 300 mL of water while volunteers in the second arm of the study took same cocktail of drugs with 300 mL of aqueous beverage of *Hibiscus sabdariffa* (as prepared in section 3.5.3.3). Volunteers fasted overnight before the commencement of the study. On the day of the study, volunteers' blood samples (5 mL) were collected at zero hour before the drugs were administered; thereafter 5 mL blood samples were taken through indwelling catheter at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, and 24 h post administration of the drugs into EDTA bottles. These were immediately centrifuged at 3500 *g* for 10 minutes. The plasma samples were then transferred into cryotubes and stored at -20^{o}C until analysed. Volunteers were given standard diet that did not interfere with the study after 5 h into the study. One week wash-out period was observed before the second phase of the study was conducted. In the second phase, the volunteers in each arm of the study were switched so that those who took the drug cocktail with water in the first phase took the drug cocktail with aqueous beverage of *Hibiscus sabdariffa* in the second phase of the study, while the volunteers in the arm that took a cocktail of caffeine 200 mg and simvastatin 40 mg with aqueous beverage of *Hibiscus sabdariffa* in the first phase took the drug cocktail with water in the second phase. Same blood sampling procedure was followed in the second phase.

3.8.5 Stock and working solutions

A 2 mg/mL stock solution of caffeine and paraxanthine, and 1 mg/mL of etofylline, the internal standard (IS), were prepared in methanol:water (50:50 v/v). Working solutions of 1.25, 2.5, 10, 20, 25, 125, 250 and 375 µg/mL of caffeine and 10, 20, 25, 50, 125, 250, and 375 µg/mL of paraxanthine were made along with a working solution of 125 µg/mL

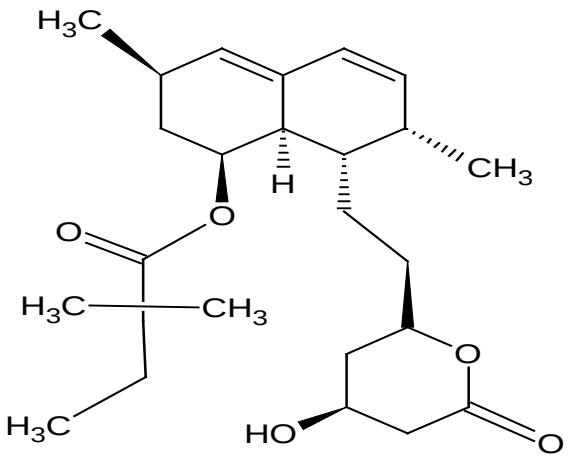
of etofylline (IS). For the analysis of simvastatin, 2 mg/mL and 1 mg/mL of simvastatin and lovastatin stock solution were respectively prepared with acetonitrile. Working solution of 75 µg/mL of lovastatin and 0.25, 0.625, 1.25, 2.5, 6.25, 12.5, 25, 62.5, 125, and 250 µg/mL of simvastatin were also prepared. Figure 3.3 shows the chemical structures of simvastatin and lovastatin (IS).

3.8.6. Sample preparation and analysis for pharmacokinetic study

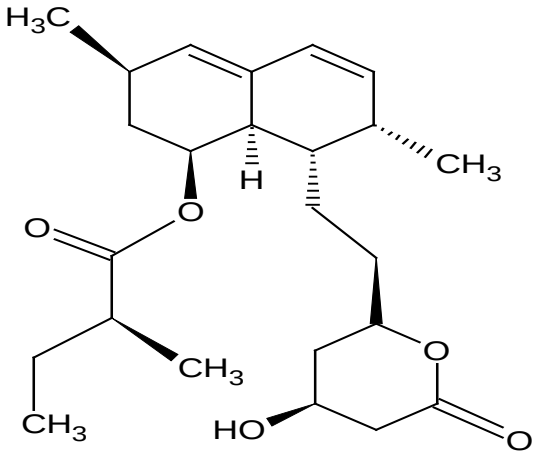
Caffeine and its metabolite paraxanthine were analysed using the method described by Uney and Tras, (2010) with slight modification. Briefly, to 250 µL of plasma was added 10 µL of 125 µg/mL of etofylline (IS). To the mixture 750 µL of ice-cold acetonitrile was then added to precipitate the protein. The mixture was vortexed for 30 sec put in the freezer for 30 min after which it was centrifuged at 5000 *g* for 10 min. The organic phase was removed and dried with nitrogen gas over water bath at 45^{o}C. The residue was reconstituted with 250 µL of the mobile phase and 20 µL injected into the column. The analytes were eluted using methanol:water (20:80 v/v) as the mobile phase at a flow rate of 1.0 mL/min. The wavelength of detection for the analytes was 280 nm.

Simvastatin was analysed by the method described by Adidala *et al* (2014) with some modifications. Summarily, 10 µL of 75 µg/mL of lovastatin was added to 250 µL of plasma, this was vortexed for 5 sec, and 750 µL of iced-cold acetonitrile was added and further vortexed for 30 sec. The mixture was put in the freezer for 30 min and then centrifuged at 5000 g for 15 min. The organic layer was separated and dried under nitrogen gas over a water bath at 45^{o}C. The dried sample was reconstituted with 250 µL of the mobile phase. Thereafter, 20 µL of the reconstituted solution was injected into the HPLC column.

The mobile phase used for the analysis of simvastatin was acetonitrile:water (80:20) pumped at a rate of 1.0 mL/min for a total run time of 6 minutes.



Simvastatin



Lovastatin

Fig 3.3: Chemical structures of simvastatin and the internal standard lovastatin

3.8.7 Calibration curves for caffeine, paraxanthine, and simvastatin

The calibration curves for caffeine and paraxanthine were generated at concentrations of 0.05, 0.1, 0.4, 0.8, 1.0, 5.0, 10.0, 15.0 µg/mL for caffeine, and 0.4, 0.8, 1.0, 2.0, 5.0, 10.0, 15.0 µg/mL for paraxanthine.

This was done by spiking blank plasma with 10 µL of the respective working solutions of caffeine and paraxanthine. The final concentration of etofylline (IS) in the plasma was 5 µg/mL when 10 µL of 125 µg/mL of etofylline was spiked into the plasma containing caffeine and paraxanthine.

Blank plasma was spiked with 10 µL of the working solutions of simvastatin to obtain final concentrations of 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1.0, 2.5, 5.0, and 10.0 µg/mL. Also 10 µL of 75 µg/mL of lovastatin (IS) was also spiked into the plasma to obtain a concentration of 3 µg/mL for the IS. Calibration curves were generated with Excel.

3.8.8 Method validation for the determination of the effect of aqueous beverage of *Hibiscus sabdariffa* on the pharmacokinetics parameters of caffeine and simvastatin

3.8.8.1 Lower limit of quantification (LLOQ)

The LLOQ for caffeine, paraxanthine and simvastatin were 0.05 µg/mL, 0.4 µg/mL, 0.01 µg/mL, respectively as determined from individual calibration standards.

3.8.8.2 Linearity

The linearity of the assay was assessed by determining the regression coefficient (R^2) for the calibration curve for caffeine, paraxanthine and simvastatin. An $R^2 > 0.99$ signified that the method was linear over the calibration standards used.

3.8.8.3 Precision and accuracy

Low quality control (LQC) concentration was determined as three times the LLOQ, the medium quality control (MQC) as 30 - 50% of the calibration range and the high quality control (HQC) as 75% of the upper limit of quantification (Food and Drug Administration, 2012; European Medicines Agency, 2014). For caffeine, the LLOQ, LQC, MQC and HQC were 0.05 µg/mL, 0.15 µg/mL, 3.5 µg/mL and 10 µg/mL respectively; while for paraxanthine, the LLOQ, LQC, MQC and HQC were 0.4 µg/mL,

1.2 µg/mL, 4.0 µg/mL and 10 µg/mL respectively. Simvastatin had 0.01 µg/mL, 0.03 µg/mL, 1.5 µg/mL and 7.0 µg/mL as the LLOQ, LQC, MQC and HQC, respectively.

Six replicates of each quality control samples were prepared and were ran at three different times of the day for the intra-day precision and accuracy determination and also three different days in the week for the determination of inter-day precision and accuracy. Precision was determined as the coefficient of variation and should not be more than 15% for the quality control sample for intra-day and inter-day runs. Accuracy was determined as the percentage of the nominal value for the quality control samples which should not be more than 15% (i.e. 85% - 115% of the nominal concentrations) for both intra-day and inter-day runs.

3.8.8.4 Percentage recovery

Recovery was determined in similar manner as described in section 3.7.1.10.3 while equation 3.6 was used to calculate the percentage recovery.

3.9 Interactions between aqueous extract of *Hibiscus sabdariffa* and simvastatin

3.9.1 Chemicals

Cholesterol powder (Oxford Chemicals Ltd, India), Mamalemon® pure vegetable oil (PZ Wilmar Foods Ltd, Nigeria), Randox kits for total cholesterol (T_c), triglyceride (TG) and high density lipoprotein cholesterol (HDL_c) (Randox Laboratories, United Kingdom) and Simvastatin (Teva, England) were used for the study. All other chemicals used were of analytical grade.

3.9.2 Determination of herb and drug dosage

Two doses of aqueous extract of *Hibiscus sabdariffa* (AEHS) and simvastatin were used based on reports from animal studies (Hirunpanich *et al*., 2006; Agoreyo *et al*., 2008). A low dose of 250 mg/kg and a high dose of 500 mg/kg body weight was used for AEHS while a low dose of 10 mg/kg and a high dose of 20 mg/kg body weight, was used for simvastatin.

3.9.3 Experimental animals

Male albino rats with weight range of 213 - 260 g were purchased from the University of Ibadan, Department of Veterinary Pathology and acclimatized for a week. They were

housed in standard plastic cages and allowed access to rat chows and water *ad libitum.* A standard guideline for the care and use of animals in research was followed throughout the study (American Psychological Association, 1986).

3.9.4 Induction of hyperlipidemia

Hyperlipidemia was induced by daily oral administration of 250 mg/kg cholesterol suspended in cholesterol-free Mamalemon® pure vegetable oil. This was administered daily in combination with a high fat diet, developed at the Department of Veterinary Medicine, University of Ibadan, containing maize reserve (46.0%), wheat bran (15.0%), soya full fat (25.4%), palm kernel cake (12.0%), bone meal (0.20%), salt (0.24%), lysine (0.24%), methionine (0.24%), premix grower (0.24%), enzymes (0.20%), limestone (0.20%), and 65.0%-fish meal (0.20%). The high fat diet was prepared by Acefeeds in Ibadan, Nigeria. Hyperlipidemia in the rats was confirmed after four weeks with a minimum of 45% increase in total cholesterol (T_c) value. Animals with confirmed hyperlipidemia were selected for the experiments, and randomly distributed into the various groups.

3.9.5 Experimental design

A 2^3 Factorial experimental design (FED) with two sets of three factors considered at two levels of high (indicated by a subscript H) and low (indicated by a subscript L) using the format described by Woolfall *et al*., (1964) and Gilbert *et al.*, (2010). The first set of factors were Nature (N_L – herb, N_H - drug), Dose (D_L – low dose, D_H – high dose), and treatment period (P_L – 2 weeks treatment period, P_H – 4 weeks treatment period). Simvastatin represented the drug while aqueous extract of *H. sabdariffa* represented the herb. The herb (AEHS) and the drug (simvastatin) were administered separately to the rats.

In the second FED, three set of factors were also considered. These were AEHS which was represented by "Z," simvastatin represented by "S" and the treatment period represented by "P." These factors were also considered at two levels which were: AEHS (Z_L – low dose, Z_H – high dose), Simvastatin (S_L – low dose, S_H – high dose) and treatment period (P_L – 2 weeks of treatment period, P_H – 4 weeks of treatment period);

AEHS and simvastatin were coadministered to groups of rats at different combination of doses for different treatment periods.

The combinations for the first set of three factors were $N_LD_LP_L$, $N_LD_LP_H$, $N_LD_HP_L$, $N_LD_HP_H$, $N_HD_LP_L$, $N_HD_LP_H$, $N_HD_HP_L$, $N_HD_HP_H$ and the combinations for the second set of three factors were $Z_LS_LP_L$, $Z_LS_LP_H$, $Z_LS_HP_L$, $Z_LS_HP_H$, $Z_HS_LP_L$, $Z_HS_LP_H$, and $Z_HS_HP_L$ and $Z_HS_HP_H$. Each combination represents a treatment group. For example $N_LD_LP_L$ represent low dose of herb given for 2-weeks. $Z_HS_HP_H$ represents high dose of AEHS plus high dose of simvastatin given for 4-weeks. A detailed description of these combinations of factors is shown in Table 3.5.

Four groups of five rats were used for each set of the FED and five rats each for the two control groups (negative and vehicle control). Body weight of each animal was measured before and after the experiment.

3.9.6 Determination of lipid profile

For the determination of lipid profile at 2 and 4 weeks of treatments, blood was collected after a 14 h overnight fast from retro-orbital sinus into heparinized tubes and centrifuged at 3000 *g* for 10 min. Plasma samples were collected and analysed using Randox® kits for total cholesterol (T_c), triglyceride (TG) and high density lipoprotein cholesterol (HDL_c). Low Density Lipoprotein cholesterol (LDL_c) value was calculated using Friedewald equation (Friedewald *et al*., 1972)

$$LDL_c(mg/dL) = \left[T_c - \left(HDL_c + \frac{TG}{5}\right)\right] \qquad \text{Equation 3.4}$$

3.10 Data analysis

3.10.1 Descriptive and inferential statistics for the survey

Data were analyzed with Statistical Package for Social Sciences (SPSS), Windows version 20.0. Descriptive statistics were used to evaluate the distribution of the participants' demographics.

Table 3.5: Description of the combination of factors in the factorial experimental designs

Factorial experimental design A		Factorial experimental design B	
Factor combinations	**Description**	**Factor combinations**	**Description**
$N_LD_LP_L$	Low dose of AEHS (250 mg/kg) given for 2-weeks	$Z_LS_LP_L$	Low dose of AEHS (250 mg/kg) + Low dose of SIM (10 mg/kg) given for 2-weeks
$N_LD_LP_H$	Low dose of AEHS (250 mg/kg) given for 4-weeks	$Z_LS_LP_H$	Low dose of AEHS (250 mg/kg) + Low dose of SIM (10 mg/kg) given for 4-weeks
$N_LD_HP_L$	High dose of AEHS (500 mg/kg) given for 2-weeks	$Z_LS_HP_L$	Low dose of AEHS (250 mg/kg) + High dose of SIM given for 2-weeks
$N_LD_HP_H$	High dose of AEHS (500 mg/kg) given for 4-weeks	$Z_LS_HP_H$	Low dose of AEHS (250 mg/kg) + High dose of SIM given for 4-weeks
$N_HD_LP_L$	Low dose of SIM (10 mg/kg) given for 2-weeks	$Z_HS_LP_L$	High dose of AEHS (500 mg/kg) + Low dose of SIM (10 mg/kg) given for 2-weeks
$N_HD_LP_H$	Low dose of SIM (10 mg/kg) given for 4-weeks	$Z_HS_LP_H$	High dose of AEHS (500 mg/kg) + Low dose of SIM (10 mg/kg) given for 4-weeks
$N_HD_HP_L$	High dose of SIM (10 mg/kg) given for 2-weeks	$Z_HS_HP_L$	High dose of AEHS (500 mg/kg) + High dose of SIM (10 mg/kg) given for 2-weeks
$N_HD_HP_H$	High dose of SIM (20 mg/kg) given for 4-weeks	$Z_HS_HP_H$	High dose of AEHS (500 mg/kg) + High dose of SIM (20 mg/kg) given for 4-weeks

AEHS – aqueous extract of *H. sabdariffa* calyces, SIM - simvastatin

Pearson Chi-square statistics and Fisher's Exact Probability test were used to detect associations between gender, self-reported indications, and side effects experienced with the usage of aqueous beverage of *Hibiscus sabdariffa.* Relative risk ratio was used to identify gender-associated self-reported side effects. Level of significance was set at p = 0.05.

3.10.2 Determination of 50% inhibitory concentration (IC_{50})

Fifty percent inhibitory concentration (IC_{50}) values were determined graphically from the logarithmic plot of inhibitor concentration (concentrations of ethanolic or aqueous extract of *Hibiscus sabdariffa*) versus percentage of enzyme activity remaining after inhibition using GraphPad Prism 5.40 software (GraphPad Software Inc., San Diego, CA). All data points represented the average of duplicate incubations. The enzyme activities in the presence of inhibitors (ethanolic or aqueous extract of *Hibiscus sabdariffa*) were compared to the vehicle incubations and positive controls. The model equation for the plot was

$$\%\ Inhibition = \frac{100}{(1 + 10^{(A - logIC_{50})})} \qquad \text{Equation 3.6}$$

Where A = log of ethanolic or aqueous extract concentration in the incubations.

Percentage Enzyme Activity Remaining (%EAR) – from LC/MS/MS readings was determined by:

$$\%\ EAR = \frac{\text{Peak area of metabolite at each concentration of the extract}}{\text{Average peak area of negative control}}\ x\ 100 \qquad \text{Equation 3.7}$$

From the plot, the following the 50% inhibitory concentration (IC_{50}) value was determined,

3.10.3 Determination of liter per dose unit

The volume to which a unit dose of the aqueous or ethanolic extract of *Hibiscus sabdariffa* should be diluted to give the same IC_{50}-value for each CYP isoform was calculated using the formula

$$IC_{50}\left(\frac{Litre}{dose}\right) = \frac{Dose\ (mg)}{IC_{50}\ (mg/L)}$$ Equation 3.8

The dose of the extract used was obtained from the report of Fakeye *et al*., (2007a). One dose unit of 300 mL of the ABHS corresponded to 2.05 g of the freeze-dried ethanolic extract or 2.75 g of freeze-dried aqueous extract of *Hibiscus sabdariffa*.

3.10.4 Classification of *in vitro* inhibitory potential of extracts of *Hibiscus sabdariffa*

The extracts of *Hibiscus sabdariffa* was classified as potent, moderate or weak *in vitro* inhibitor of CYP isoforms depending on the IC_{50} value obtained (Gwaza *et al.*, 2009; Kong *et al.*, 2011). Potent inhibitors have $IC_{50} \leq 10$ µg/mL, moderate inhibitors have $IC_{50} > 10$ µg/mL and ≤ 100 µg/mL and weak inhibitors have $IC_{50} > 100$ µg/mL.

3.10.5 Determination of individual coefficient of factors

Percentage decrease in T_c, TG, LDL_c, and percentage increase in HDL_c were used to determine individual and interaction coefficients using Woolfall analysis (Woolfall, 1964) and factorial experimental analysis with the aid of Minitab© 14.2. For example, the individual effect of nature (N) on each lipid parameter was determined by finding the average of the sum of treatment groups with N_H minus sum of treatment groups with N_L. This helped in quantifying the effect of changing the factor "Nature-N" from "low" to "high." The same was done for dose (D) and treatment period (P).

$$\text{Individual coefficient of N} = \left[\frac{(N_H D_L P_L + N_H D_L P_H + N_H D_H P_L + N_H D_H P_H) - (N_L D_L P_L + N_L D_L P_H + N_L D_H P_L + N_L D_H P_H)}{4}\right]$$

Equation 3.9

A positive or negative coefficient value indicated an increase or a decrease in the lipid profile value respectively. Thus, a positive coefficient value for factor "N" for any of the lipid profile parameters except HDL_c meant that changing the nature of the substance administered to the rats from "low" (represented by the herb) to "high" (represented by the drug) caused a further reduction in the respective lipid profile. This invariably meant that the drug (N_H) is more effective in lowering the respective lipid profile than the herb (N_L). The same interpretation is given to other factors. The level of significance for each individual coefficient was set at p=0.05

3.10.6 Determination of interaction coefficient between factors

For the interaction effects between factors (N-D, N-P, and D-P), a coefficient value of zero indicated no interaction. A positive or negative value indicated an additive or opposing interaction effect, respectively (Fakeye *et al*., 2007b). For example, the interaction effect between Nature and Dose, N-D, was determined by finding the average of the sum of all treatment groups with N_HD_H and N_LD_L components minus the sum of all other treatment groups.

$$\text{Interaction coefficient of N} - \text{D} = \left[\frac{(N_LD_LP_L + N_LD_LP_H + N_HD_HP_L + N_HD_HP_H) - (N_HD_LP_L + N_HD_LP_H + N_LD_HP_L + N_LD_HP_H)}{4}\right]$$

Equation 3.10

Similar calculation was done to determine the interaction coefficient for other interacting variable like N-P, and D-P. Where interaction between two factors is significant, the significant individual effects were ignored and the simple main effect was reported. The simple main effect showed how one level of a factor interacted with the other levels in the second factor to influence the value of the lipid profile. An interaction depicts this.

Analysis of variance with Least Significance Difference (LSD) *post hoc* test was used to determine difference between individual administration of AEHS and simvastatin and concomitant administration. Significant difference were set at $p<0.05$.

3.10.7 Determination of phenotypic metabolic ratio

Phenotypic metabolic ratios (PMR) of caffeine and metoprolol were determined using equations 3.11 and 3.12, respectively.

$$PMR\ of\ Caffeine = \frac{Mean\ Concentration\ of\ Paraxanthine}{Mean\ Concentration\ of\ Caffeine}$$

Equation 3.11

$$PMR\ of\ Metoprolol = \frac{Mean\ Concentration\ of\ Metoprolol}{Mean\ Concentration\ of\ \propto -OH\ Metoprolol}$$

Equation 3.12

The significant difference in the PMR when these drugs were administered with water and with aqueous beverage of *Hibiscus sabdariffa* was determined using paired sample t-test with the significance level set at $p<0.05$. A significant difference signifies that the

extract is an inhibitor of the CYP isoform that metabolises the drugs; CYP1A2 for caffeine and CYP2D6 for metoprolol.

3.10.8 Pharmacokinetic analysis

The pharmacokinetic parameters were determined with PKsolver® compartmental analysis software, version 2.0. The parameters evaluated included, the area under the plasma concentration-time curves (AUC_{0-24}, and $AUC_{0-\infty}$), the peak plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}). The apparent total body clearance (CL/F), apparent volume of distribution (V/F), absorption rate constant (K_a), elimination rate constant (K_{el}) were also determined. The corresponding elimination half-life ($t_{½}K_e$) and absorption half-life ($t_{½}K_a$) were also estimated. Significant changes in these parameters when the drug cocktail of caffeine and simvastatin were administered to the volunteers with aqueous beverage of *Hibiscus sabdariffa* and with water were determined using paired sample t-test with level of significance set at p = 0.05. According to European Medicine Agency, (2014), the magnitude of the differences in the pharmacokinetic parameters of the drugs (caffeine and simvastatin) when coadministered with or without the extract may be more important than p-values, thus, the determination of the fold-change as calculated below (Equation 3.13) is important for parameters like AUC, C_{max}, K_a, K_e CL/F and $t_{1/2}$.

$$Fold - change = \frac{Mean\ value\ of\ drug\ PK\ parameter\ when\ coadministered\ with\ extract}{Mean\ value\ of\ drug\ PK\ parameter\ when\ coadministered\ with\ water}$$

Equation 3.13

3.10.9 Determination of *in vivo* inhibitory potential of aqueous beverage of *Hibiscus sabdariffa*

According to European Medicine Agency, (2014) the *in vivo* inhibitory potential of aqueous beverage of *Hibiscus sabdariffa* on CYP1A2 and CYP3A4 isoforms can be categorised by comparing the $AUC_{0-\infty}$ when the CYP substrates, caffeine or simvastatin, were administered with water and with aqueous beverage of *Hibiscus sabdariffa*. The fold-change in the $AUC_{0-\infty}$ determines the level of inhibitory potential of aqueous beverage of *Hibiscus sabdariffa*:

- $AUC_{0-\infty} \geq 5$ folds – indicate that the extract is a strong inhibitor
- $AUC_{0-\infty} \geq 2$ and ≤ 5 fold – indicate that the extract is a moderate inhibitor
- $AUC_{0-\infty} \geq 1.25$ and ≤ 2 fold – indicate that the extract is a weak inhibitor.

CHAPTER FOUR

RESULTS

4.1 Percentage yield and anthocyanin content of *Hibiscus sabdariffa* extracts

The percentage yield of ethanolic and aqueous extracts of *Hibiscus sabdariffa* were 25.0% and 33.6% respectively. The total monomeric anthocyanin content for ethanolic and aqueous extracts of *Hibiscus sabdariffa* as Cyanidin-3-glucoside were 58.3 mg/100 g and 36.4 mg/100 g of the dried powder, respectively. The phytochemicals present in powdered calyces of *Hibiscus sabdariffa* were alkaloids, cardenolides, saponins, tannins, and flavonoids. Also, the pH of ethanolic extract, aqueous extract, and aqueous beverage of *Hibiscus sabdariffa* were 3.43, 2.67, and 2.50, respectively.

4.2 Pattern and extent of use of the aqueous beverage of *Hibiscus sabdariffa*

The age range of the participants was 16 to 65 years with a mean age of 26.67 ± 9.79 years. Response rate for thisrvey was 87.2%. Three hundred and ninety eight (30.4%) participants were staff. Male participants were 613 (49.3%). Other demographic characteristic of the participants are shown in Table 4.1. The number of people who had taken aqueous beverage of *Hibiscus sabdariffa* (ABHS) prior to the study was 1267 (96.9%). About 40 (3.1%) of the participants never took ABHS.

Frequency of consumption of ABHS as described by the participants were; daily 38 (2.9%), once a week 128 (9.8%), twice a week 89 (6.8%) and occasionally 1012 (77.4%). Majority of people, 1209 (92.4%) took ABHS as beverage while 32 (2.4%) took it as alcoholic drink. The various ways of flavouring the drink were with sugar 735 (56.2%), garlic 235 (18.0%), ginger 608 (46.5%), honey 315 (24.1), artificial sweeteners 117 (8.9%), and clove 2 (0.2%).

Table 4.1: Demographic characteristics of participants in the study of extent and pattern of use of aqueous beverage of *Hibiscus sabdariffa*

Variables	Frequency (%)
Sex (n = 1243)	
Male	613 (49.3)
Female	630 (50.7)
Status (n = 1308)	
Staff	398 (30.4)
Student	910 (69.6)
Educational level (n = 1253)	
Primary	11 (0.9)
Secondary	895 (71.4)
Tertiary	347 (27.7%)
Marital status (n = 1265)	
Unmarried	940 (74.3)
Married	313 (24.7)
Divorced	4 (0.3)
Separated	4 (0.3)
Widowed	4 (0.3)
Religion (n = 1269)	
Christianity	1058 (83.4)
Islam	207 (16.3)
Traditional	4 (0.3)

Participants' reasons for taking ABHS were: for relaxation 382 (29.2%), to lower blood pressure 236 (18.0%), to lower cholesterol level 181 (13.8%), to lower glucose level 151 (11.5%) and other reasons listed in Table 4.2.

Students use ABHS more than staff as a form of relaxant, $p = 0.002$, (Table 4.2) while staff use ABHS to treat diseases like hypertension, $p = 0.001$, and to lower cholesterol level, $p = 0.045$, (Table 4.2) more than students.

Participants who reported experiencing side effects after taking *Zobo* were 210 (16.1%). Some of these side effects are shown in Table 4.3 and included diarrhoea 69 (5.3%), dizziness 41 (3.1%), tiredness 29 (2.2%) and blurred vision 16 (1.2%). There were no significant differences among staff and students with respect to side effects experienced except for stomach upset (Table 4.3).

About one-eighth of the participants 164 (12.5%) claimed they sometimes used ABHS to take their medicines. The classes of drugs coadministered with ABHS were analgesics 71 (5.4%), antibiotics 51 (3.9%), antihypertensives 24 (1.8%), antilipidemics 16 (1.2%), oral hypoglycemic agents 13 (1.0%) and others listed in Table 4.4. About 93 (56.7%) of the participants who coadministered ABHS with other drugs were on chronic medication and the medicines taken daily by these participants included Moduretic® (amiloride and hydrochlorothiazide), Nifedipine, Vasoprin® (low-dose aspirin), Felxicam® (piroxicam), Atenolol, Lisinopril Omeprazole, Amlodipine, Septrin® (cotrimoxazole), Ramipril, Ibuprofen, Glanil® (glibenclamide), Glucophage® (metformin) and Micardis® (telmisartan).

Of the 93 (56.7%) participants who were on chronic medications, 18 (19.4%) reported that concomitant administration of ABHS with their medications had caused them some discomfort which included stomach-ache 4 (22.2%), diarrhoea 3 (16.7%); dizziness one (5.6%) and headache one (5.6%). Less than half of these discomforts 7 (38.9%), had resulted in hospitalisation. The duration of hospitalisation was not ascertained in the study. One-third of the participants, 453 (34.6%), considered ABHS drink as medicinal.

Table 4.2: Differences between staff and students on the reasons why people take aqueous beverage of *Hibiscus sabdariffa*

Indications	Frequency (%)			P-value (2-tailed)[a]
	Total	Staff	Students	
As a beverage	706 (54.0)	186 (46.7)	520 (57.1)	0.001*
Source of vitamin C	440 (33.6)	146 (36.7)	294 (32.3)	0.127
As a relaxant	382 (29.2)	94 (23.6)	290 (31.9)	0.002*
Hematinic	318 (24.3)	105 (26.4)	213 (23.4)	0.263
To lower blood pressure	236 (18.0)	97 (24.4)	139 (15.3)	0.001*
To improve vision	223 (17.0)	57 (14.3)	119 (13.1)	0.539
To reduce blood cholesterol level	181 (13.8)	67 (16.8)	114 (12.5)	0.045*
To aid sleep	164 (12.5)	67 (16.8)	97 (10.7)	0.003*
To lower blood glucose level	151 (11.5)	50 (12.6)	101 (11.1)	0.453
For weight reduction	140 (10.7)	40 (10.1)	100 (11.0)	0.697
To relief constipation	139 (10.6)	47 (11.8)	92 (10.1)	0.380
To improve fertility	127 (9.7)	51 (12.8)	76 (8.4)	0.015*
To heal haemorrhoid	87 (6.7)	31 (7.8)	56 (6.2)	0.279
To cure liver disease	87 (6.7)	33 (8.3)	54 (5.9)	0.118
To cure kidney disease	84 (6.4)	29 (7.3)	48 (5.3)	0.161
To relief ulcer pain	84 (6.4)	29 (7.3)	55 (6.0)	0.393
To improve sexual performance	77 (5.9)	32 (8.0)	45 (4.9)	0.040*
To relief dysmenorrhea	61 (4.7)	18 (4.5)	40 (4.4)	0.885
To regulate irregular menstruation	61 (4.7)	22 (5.5)	39 (4.3)	0.322
To ease child birth	47 (3.6)	22 (5.5)	25 (2.7)	0.016*

*P<0.05, [a]Chi-square test.

Table 4.3: Differences between staff and students on side effects experienced with aqueous beverage of *Hibiscus sabdariffa*

Side effects	Frequency (%)			Relative	95% confidence	P-value 2-tailed[a]
	Total	Staff	Student	risk ratio	interval	
Diarrhoea	69 (5.3)	21 (5.3)	48 (5.3)	1.000	0.607 to 1.648	1.000
Excessive menstruation	44 (3.4)	15 (3.8)	29 (3.2)	1.183	0.641 to 2.181	0.618
Dizziness	41 (3.1)	13 (3.3)	28 (3.1)	1.062	0.556 to 2.028	0.864
Vomiting	40 (3.1)	10 (2.5)	30 (3.3)	0.762	0.376 to 1.544	0.491
Tiredness	29 (2.2)	11 (2.8)	18 (2.0)	1.397	0.666 to 0.415	0.415
Insomnia	27 (2.1)	8 (2.0)	19 (2.1)	0.963	0.425 to 2.181	1.000
Decreased libido	24 (1.8)	10 (2.5)	14 (1.5)	1.633	0.732 to 3.645	0.263
Headache	19 (1.5)	5 (1.3)	14 (1.5)	0.817	0.296 to 2.252	0.806
Blurred vision	16 (1.2)	8 (2.0)	8 (0.9)	2.286	0.864 to 6.049	0.102
Menstrual Irregularity	15 (1.1)	7 (1.8)	8 (0.9)	2.001	0.730 to 5.479	0.170
Stomach upset	12 (0.9)	0 (0.0)	12 (1.3)	-	-	0.023*
Throat irritation	6 (0.5)	0 (0.0)	6 (0.7)	-	-	0.186
Black/greyish faeces	6 (0.5)	1(0.3)	5 (0.5)	0.457	0.054 to 3.901	0.674
Excessive urination	3 (0.2)	0 (0.0)	3 (0.3)	-	-	0.558
Discolouration of tongue	2 (0.2)	1 (0.3)	1 (0.3)	2.286	0.143 to 36.463	0.516
Excessive sleep	1 (0.1)	1 (0.3)	0 (0.0)	-	-	0.304
Chest pain	1 (0.1)	0 (0.0)	1 (.10)	-	-	1.000

*P<0.05, [a]Chi-square test or Fisher Exact Probability test

Table 4.4: Classes of drugs coadministered with aqueous beverage of *Hibiscus sabdariffa* by participants

Class of drugs	Frequency %		
	Total	**Staff**	**Students**
Analgesics	71 (5.4)	23 (5.8)	48 (5.3)
Antimalarias	69 (5.3)	22 (5.5)	47 (5.2)
Antibiotics	51 (3.9)	9 (2.3)	42 (4.6)
Antihypertensives	24 (1.8)	14 (3.5)	10 (1.1)
Antiulcer	19 (1.5)	7 (1.8)	12 (1.3)
Antipsychotics	18 (1.4)	8 (2.0)	10 (1.1)
Drugs for haemorrhoids	18 (1.4)	8 (2.0)	10 (1.1)
Antilipidemic	16 (1.2)	9 (2.3)	7 (0.8)
Cardiovascular disease drugs	16 (1.3)	10 (2.5)	6 (0.7)
Drugs for asthma	15 (1.1)	8 (2.0)	7 (0.8)
Antihyperglycemic agents	13 (1.0)	4 (1.0)	9 (1.0)
Antituberculous drugs	11 (0.8)	5 (1.3)	6 (0.7)
Antiretroviral	11 (0.8)	5 (1.3)	6 (0.7)

4.3 Inhibitory effect of the extracts of *Hibiscus sabdariffa* on cytochrome P450

No matrix effect was observed at concentrations at or below 100 µg/mL for any of the probe metabolites. Peak area of 7-hydroxycoumarin was 74% when 1000 µg/mL of extract was added into control incubation. The peak areas for 7-hydroxycoumarin at 1000 µg/mL was corrected based on the observed relative matrix effect in comparison to control incubations by multiplying the observed peak areas with the observed matrix suppression factor (see Appendix G).

A plot of the percentage enzyme inhibition against the different concentrations of ethanolic extract of *Hibiscus sabdariffa* (EEHS) showed inhibitory activities of the extract on the eight CYP isoforms screened, namely: 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4 (Table 4.5). A sample chromatogram of the probe substrates metabolites monitored is shown in Fig 4.1.

Ethanolic extract of *Hibiscus sabdariffa* inhibited the formation of the metabolites of CYP isoform probe substrates previously outlined in Table 3.1. The IC_{50} values in µg/mL ranged from 306 µg/mL for CYP1A2 to 1660 µg/mL for CYP2A6 (Table 4.5), while the IC_{50} values calculated in Litre/unit dose ranged from 1.2 Liter for CYP2A6 to 7.1 Liter for CYP1A2. The order of inhibition of the investigated CYP isoforms by EEHS was 1A2 > 2C8 > 2D6 > 2B6 > 2C19 > 3A4 >> 2C9 >> 2A6.

Aqueous extract of *Hibiscus sabdariffa* (AEHS) also inhibited the formation of the metabolites of CYP isoforms probe substrates outlined in Table 3.2. Figures 4.2 to 4.9 show a non-linear logarithmic plot of percentage enzyme activity remaining against the concentration of ethanolic or aqueous extracts of *Hibiscus sabdariffa.* Most inhibition above 50% by the extracts occur at higher concentrations of the extracts.. The IC_{50} values for AEHS ranged from 399 µg/mL for CYP2C8 to 4977 µg/mL, for CYP3A4, (Table 4.5). The extract inhibited CYP2C8, CYP1A2 and CYP2B6 by 80%, 76% and 63% respectively at 1000 µg/mL (Fig 4.10).

Table 4.5: IC_{50}-values for the *in vitro* inhibition of eight CYP isoforms by the extracts of the calyces of *Hibiscus sabdariffa*

CYP isoforms	Substrates	IC_{50} values for EEHS (µg/mL)	IC_{50} values for AEHS (µg/mL)	Positive control	Positive control IC_{50} values (µg/mL)
1A2	Melatonin	306		Fluvoxamine	0.026
	Phenacetin		517		
2A6	Coumarin	1660	1448	Tranylcypromine	0.365
2B6	Bupropion	481	535	Ticlopidine	0.026
2C8	Amodiaquine	424		Quercetin	17.47
	Repaglinide		399		
2C9	Tolbutamide	744		Sulphaphenazole	0.063
	Diclofenac		1754		
2C19	Omeprazole	621	2319	Fluconazole	1.746
2C19	Omeprazole	546	1723		1.96
2D6	Dextromethorphan	446	1135	Quinidine	0.011
3A4	Midazolam	589	4977	Ketoconazole	0.957
	Testosterone	1307	1699		0.037
	Omeprazole	633	1978		0.043
	Omeprazole	600	1539		0.069

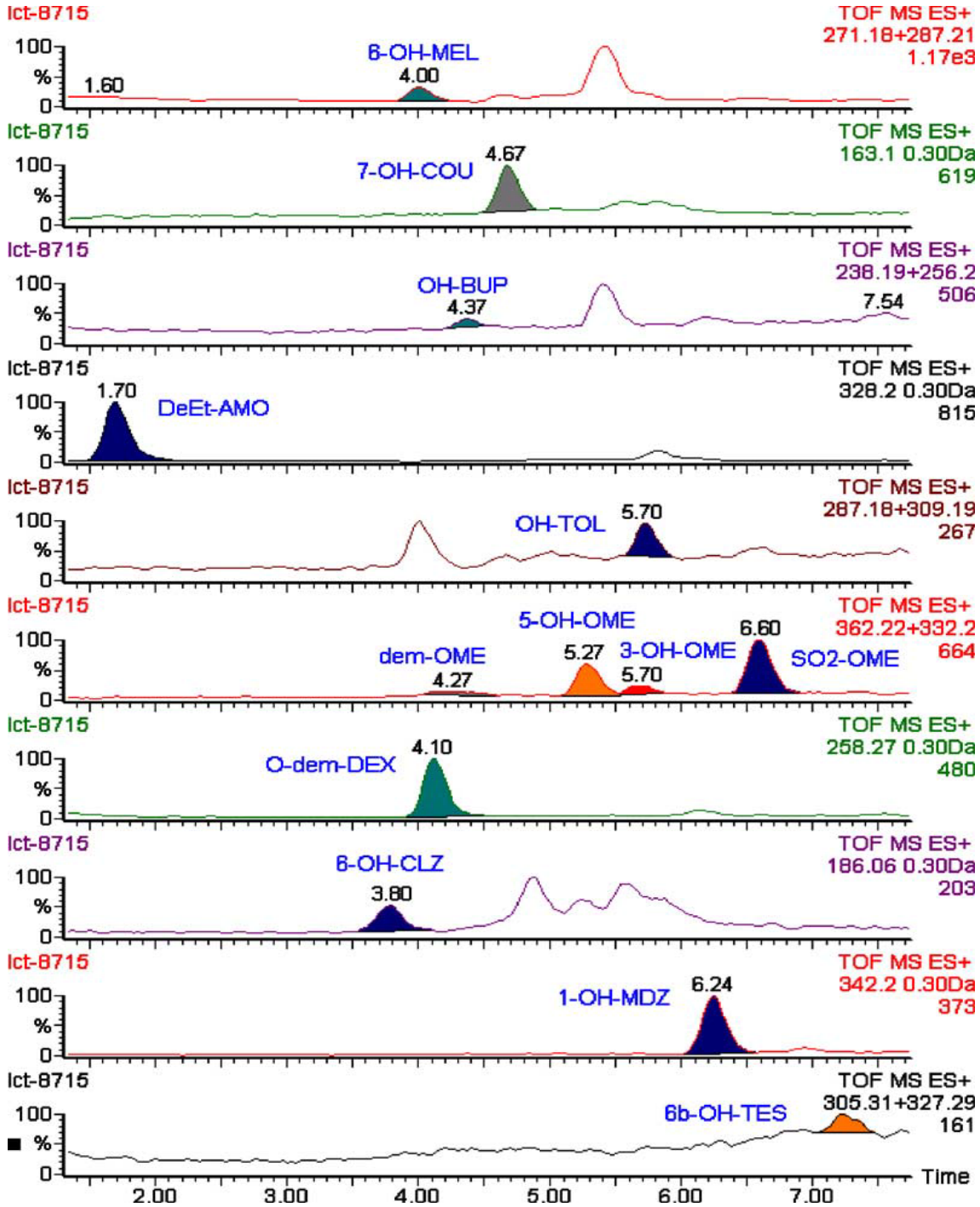


Fig 4.1: Mass chromatograms of probe metabolites after 30 min cocktail incubation for both ethanolic and aqueous extract of *Hibiscus sabdariffa*

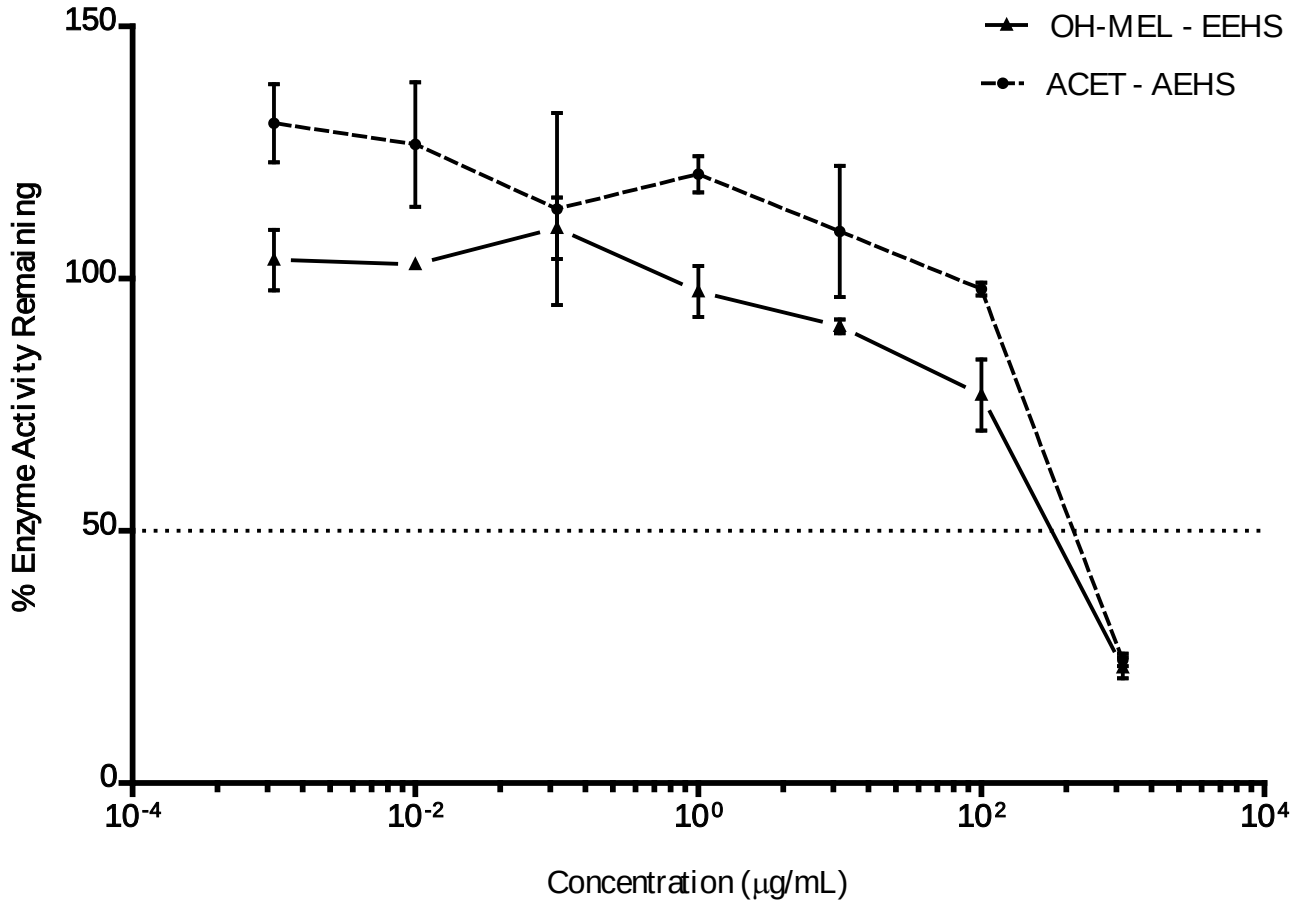


Fig 4.2 Inhibition of CYP1A2 isoform activity by ethanolic and aqueous extracts of *Hibiscus sabdariffa* (EEHS and AEHS) on the formation of acetaminophen (ACET) and hydroxymelatonin (OH-MEL). Mean and standard error of duplicate experiments are shown and 50% enzyme inhibition concentration (IC_{50}) is shown by the dotted line.

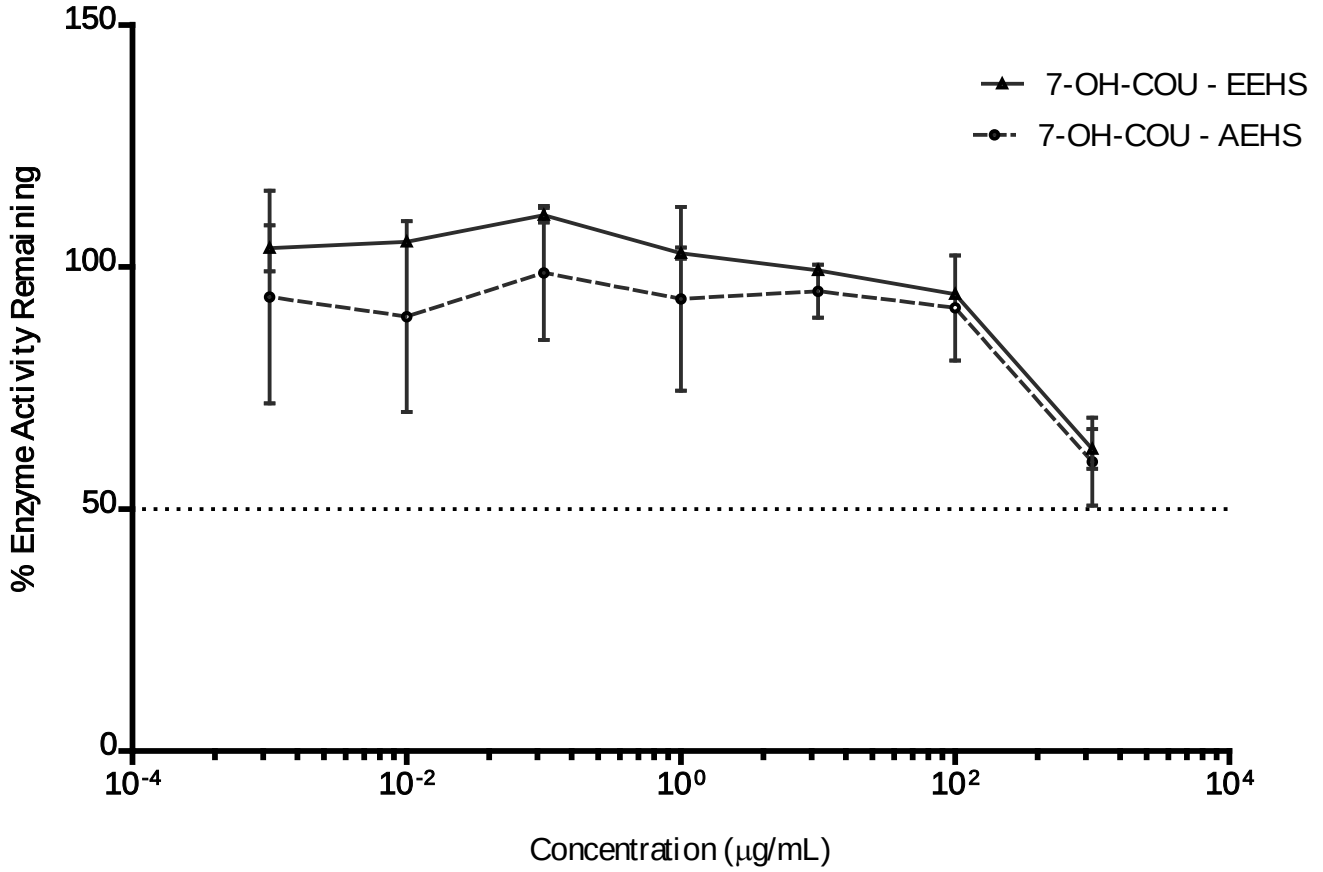


Fig 4.3 Inhibition of CYP2A6 isoform activity by ethanolic and aqueous extracts of *Hibiscus sabdariffa* (EEHS and AEHS) on the formation of 7-hydroxy-coumarin (7-OH-COU). Mean and standard error of duplicate experiments are shown and the 50% enzyme inhibition concentration (IC_{50}) is shown by the dotted line.

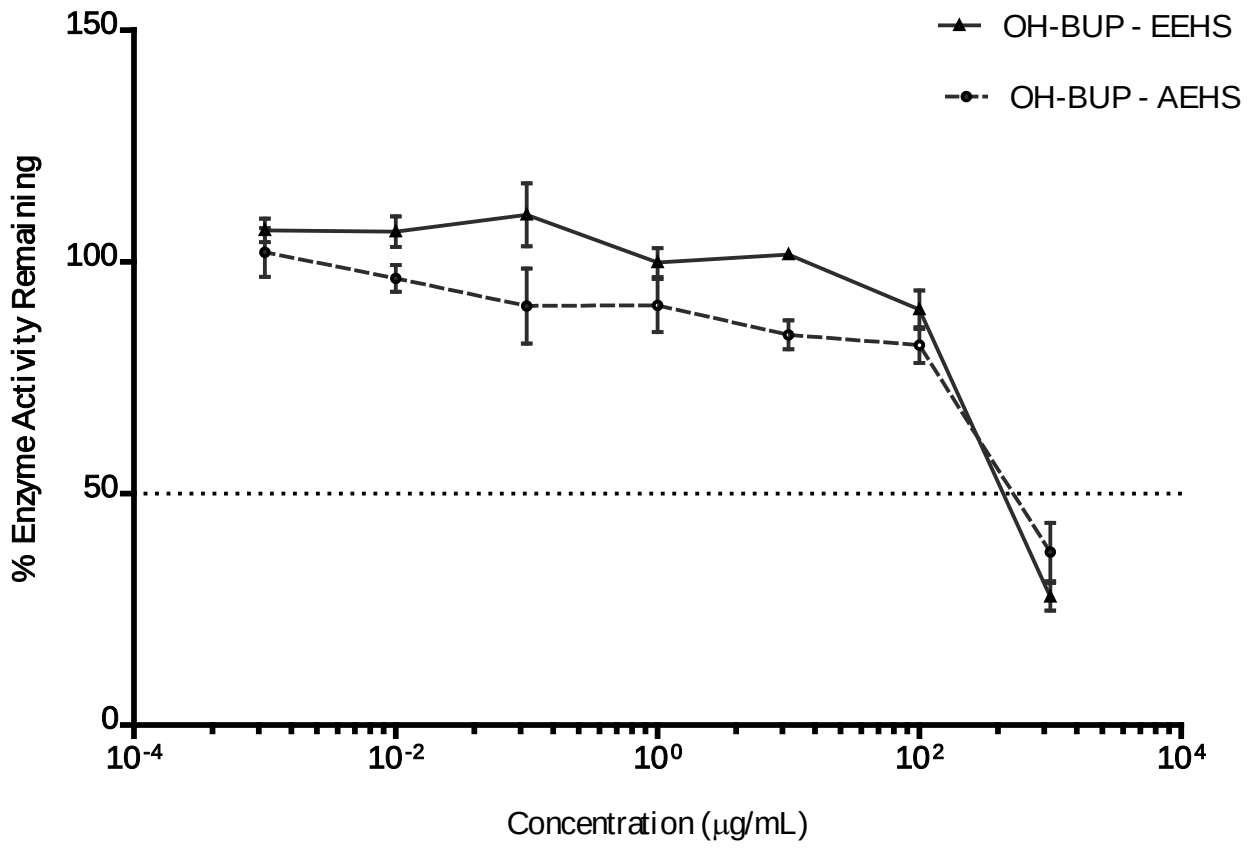


Fig 4.4 Inhibition of CYP2B6 isoform activity by ethanolic and aqueous extracts of *Hibiscus sabdariffa* (EEHS and AEHS) on the formation of hydroxy-bupropion (OH-BUP). Mean and standard error of duplicate experiments are shown and the 50% enzyme inhibition concentration (IC_{50}) is shown by the dotted line.

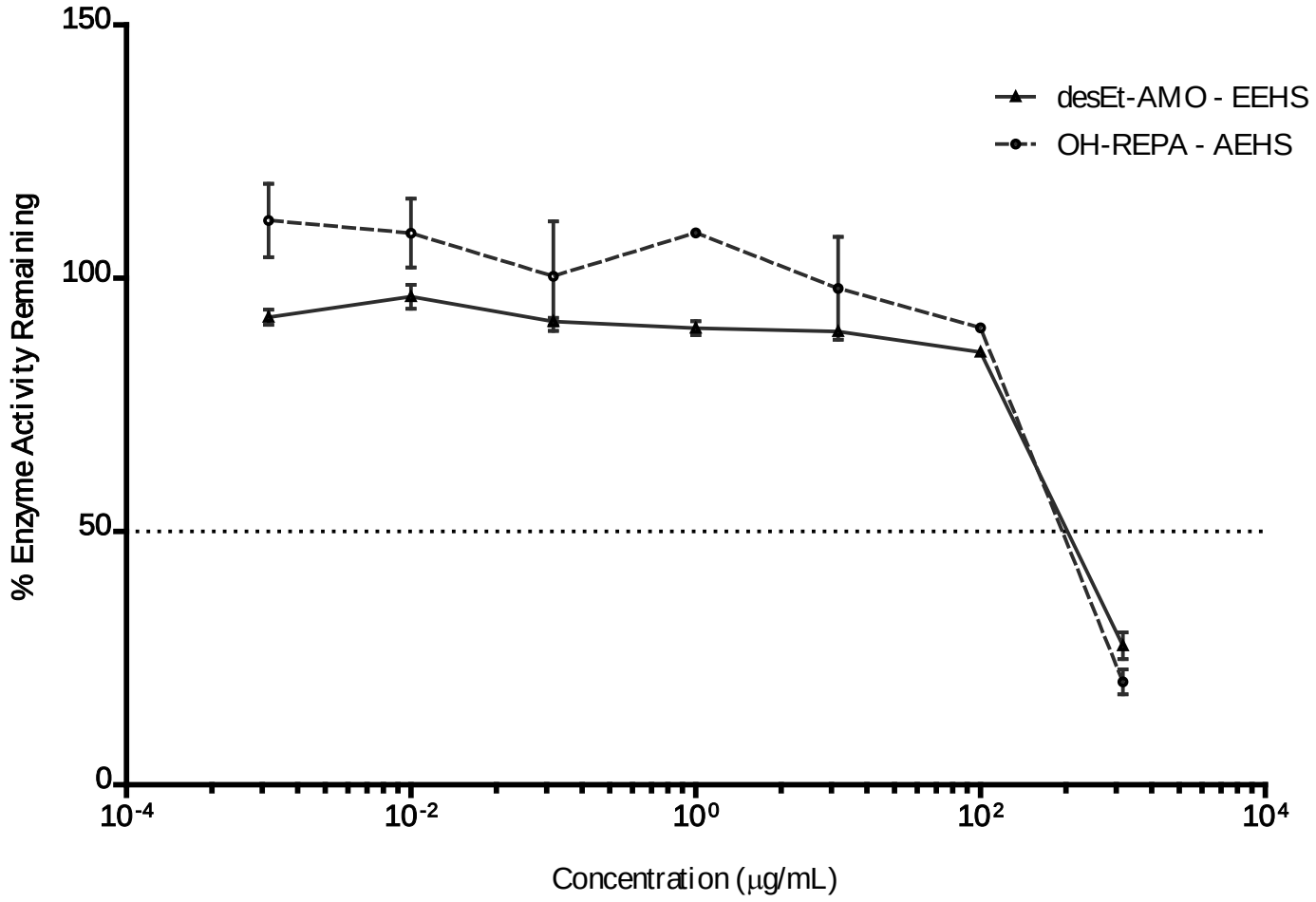


Fig 4.5 Inhibition of CYP2C8 activity by ethanolic and aqueous extracts of *Hibiscus sabdariffa* (EEHS and AEHS) on the formation of hydroxyl-repaglinide (OH-REPA) and desethylamodiaquine (desEt-AMO). Mean and standard error of duplicate experiments are shown and the 50% enzyme inhibition concentration (IC_{50}) is shown by the dotted line.

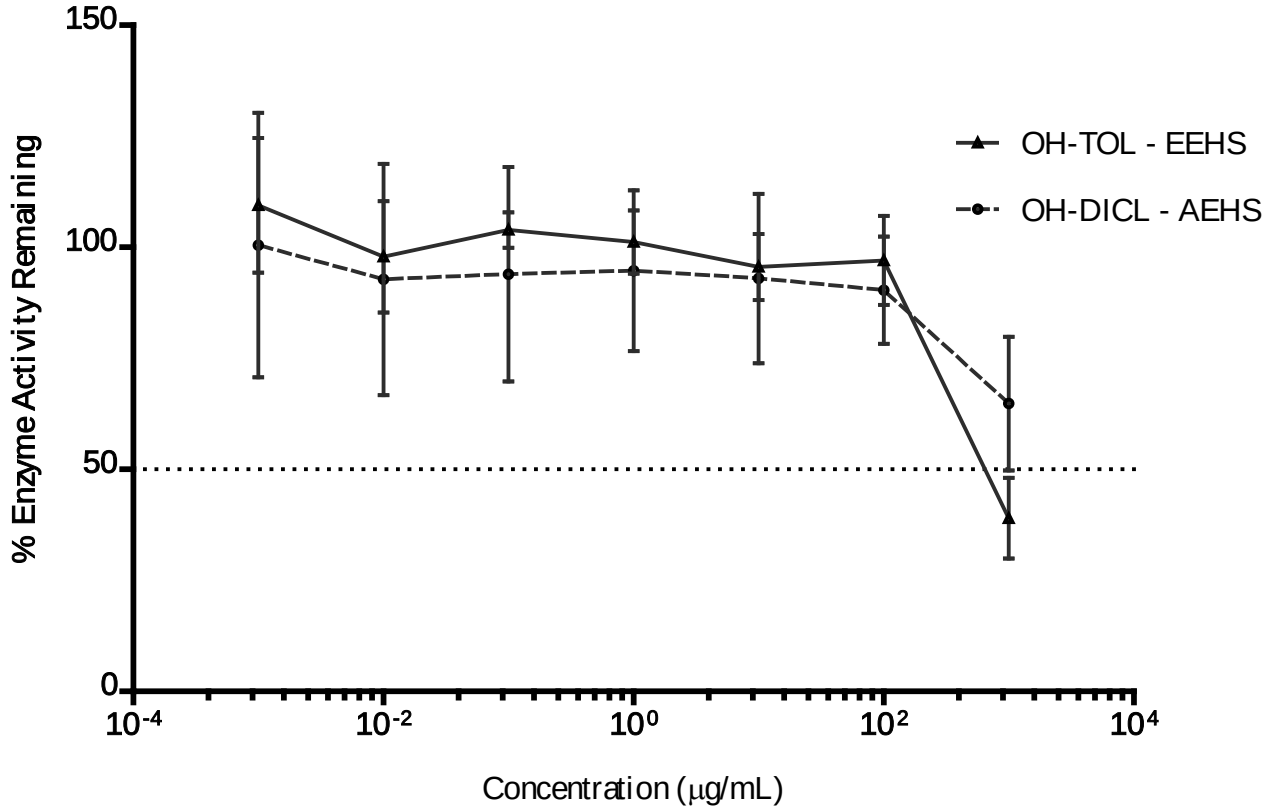


Fig 4.6 inhibition of CYP2C9 activity by ethanolic and aqueous extracts of *Hibiscus sabdariffa* (EEHS and AEHS) on the formation of 4-hydroxy-diclofenac (4-OH-DICL) and hydroxyl-tolbutamide (OH-TOL). Mean and standard error of duplicate experiments are shown and the 50% enzyme inhibition concentration (IC_{50}) is shown by the dotted line.

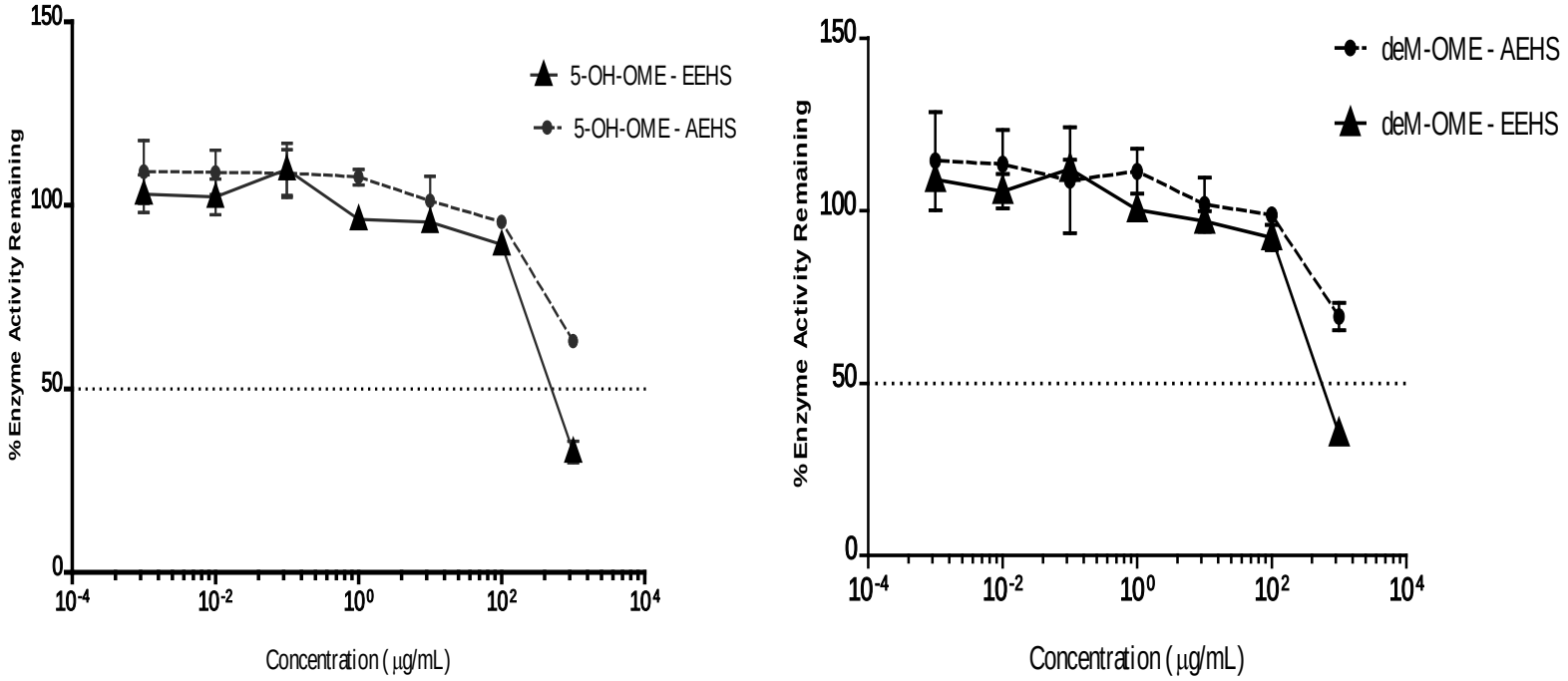


Fig 4.7 Inhibition of CYP2C19 isoform activity by ethanolic and aqueous extracts of *Hibiscus sabdariffa* (EEHS and AEHS) on the formation of 5-hydroxy-omeprazole (5-OH-OME) and desmethyl-omeprazole (deM-OME). Mean and standard error of duplicate experiments are shown and the 50% enzyme inhibition concentration (IC_{50}) is shown by the dotted line.

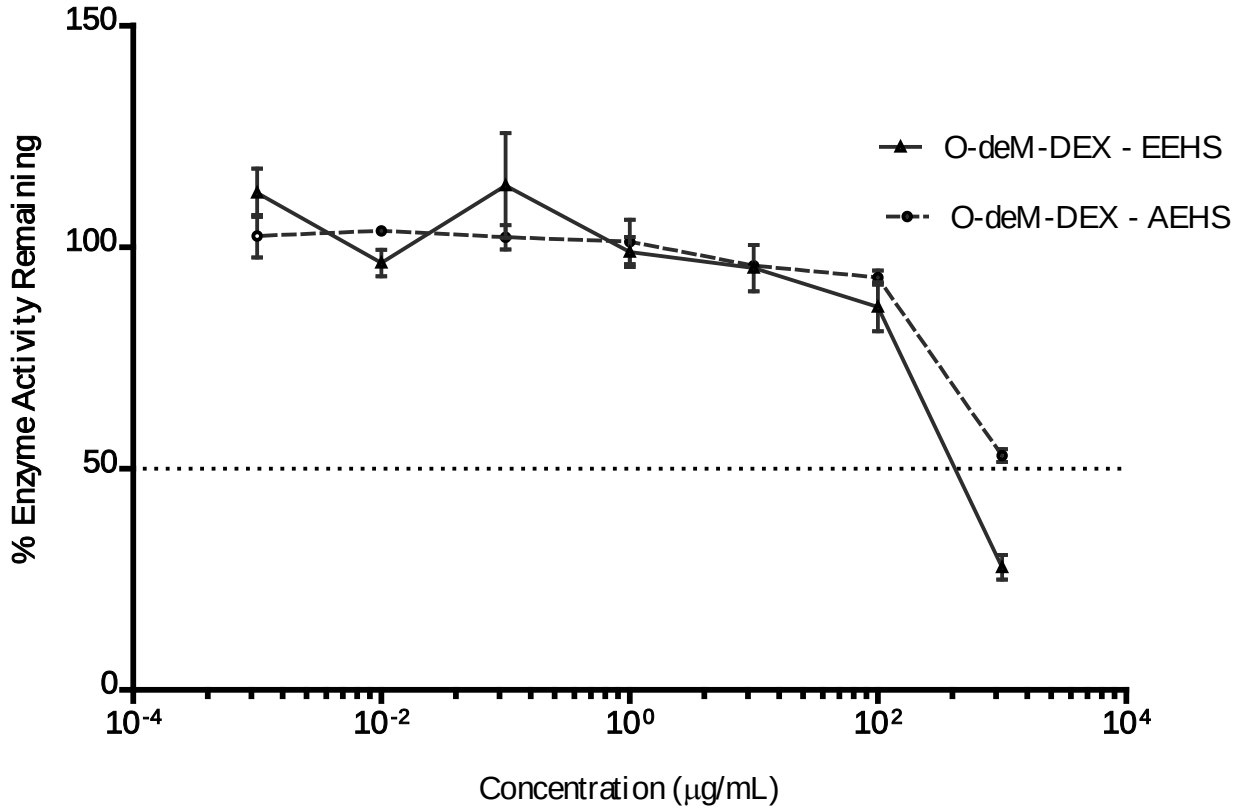


Fig 4.8 Inhibition of CYP2D6 isoform activity by ethanolic and aqueous extracts of *Hibiscus sabdariffa* (EEHS and AEHS) on the formation of dextrorphan (O-deM-DEX). Mean and standard error of duplicate experiments are shown and the 50% enzyme inhibition concentration (IC_{50}) is shown by the dotted line.

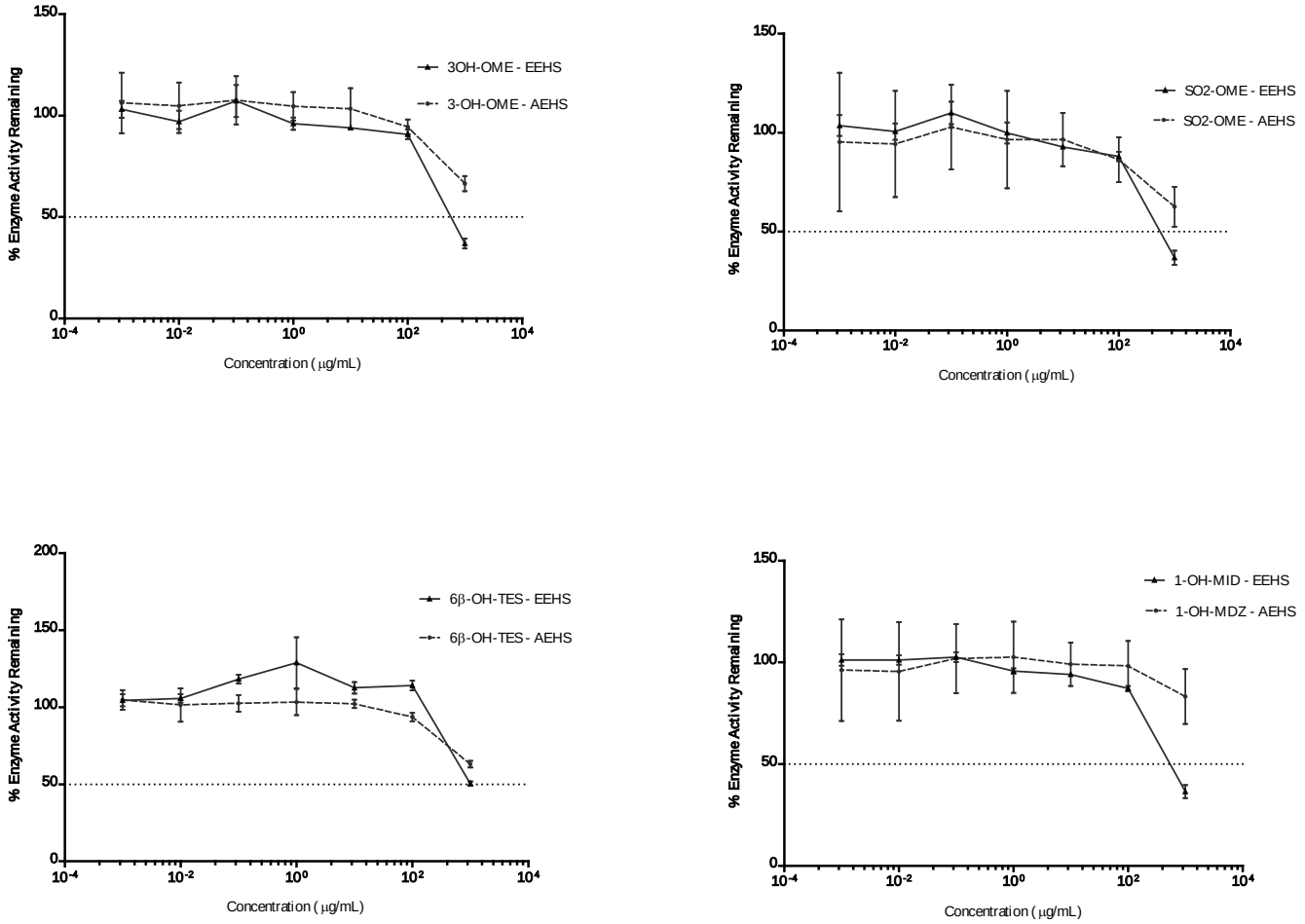


Fig 4.9 Inhibition of CYP3A4 isoform activity by ethanolic and aqueous extracts of *Hibiscus sabdariffa* (EEHS and AEHS) on the formation of 1-hydroxy-midazolam (1-OH-MDZ), 6β-hydroxy-testosterone (6β-OH-TES), omeprazole sulphone (SO_2-OME), and 3-hydroxy-omeprazole (3-OH-OME). Mean and standard error of duplicate experiments are shown and the 50% enzyme inhibition concentration (IC_{50}) is shown by the dotted line.

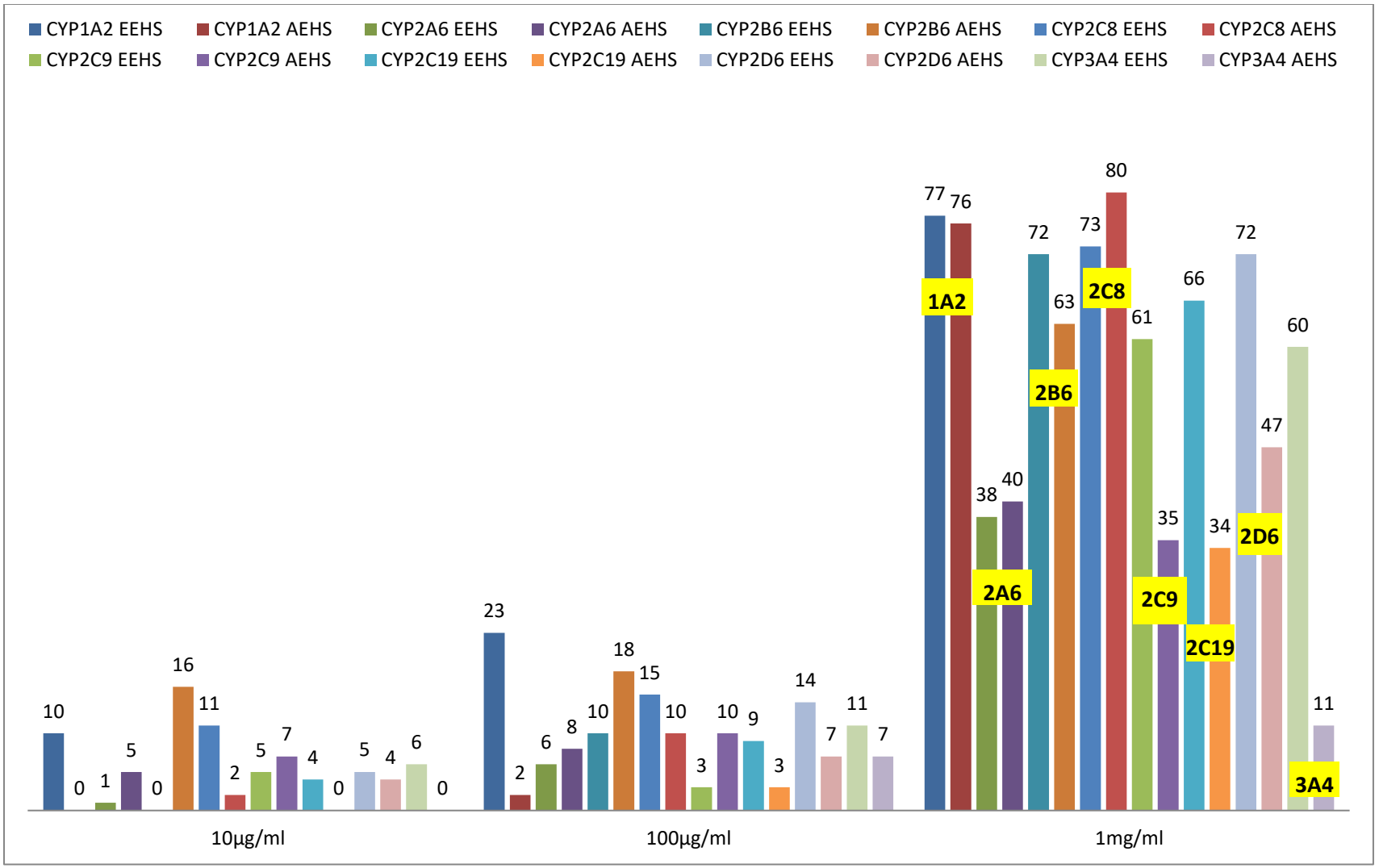


Fig 4.10: Percentage inhibition of cytochrome P450 by ethanolic and aqueous extracts of *Hibiscus sabdariffa in vitro*. Each bar represents an average of replicate incubation. For CYP2C19 and CYP3A4, average values of all the metabolites monitored are shown

The order of inhibition by aqueous extract of *Hibiscus sabdariffa* on the selected CYP isoforms was 2C8 > 1A2 > 2B6 >> 2D6 > 2A6 > 2C9 > 2C19 > 3A4. The IC_{50} values in litre per dose ranged from 1.30 L to 6.88 L (Fig 4.11). Aqueous and ethanolic extracts of *Hibiscus sabdariffa* weakly inhibited the eight selected CYP isoforms with IC_{50} values greater than 100 μg/mL.

4.4 *In vivo* inhibitory activity of aqueous beverage of *Hibiscus sabdariffa* on CYP1A2 and CYP2D6 isoforms.

The demographic characteristics, hematological and biochemical indices of volunteers in caffeine and metoprolol analysis are within normal range (see Appendix H). Calibration ranges for caffeine and paraxanthine were linear over 1 – 100 μg/mL and 0.05 – 100 μg/mL, respectively; and for metoprolol and α-hydroxymetoprolol it was linear over 10 - 1000 ng/mL. Back-calculated concentrations of the calibration standards were within ±15% of the nominal values and ±20% for LLOQ. More than 75% of the calibration standards of each analyte fulfilled this. The calibration curves for the determination of caffeine, paraxanthine, metoprolol, and α-hydroxymetoprolol concentrations in the plasma samples were linear with R^2 >0.99 (see Appendix I to L).

Under the chromatographic conditions, the analytes peaks and the internal standard peaks were well resolved. There were no interfering peaks in the chromatograms for blank sample (processed matrix without IS and analytes) and zero sample (processed matrix with only IS) (Appendix M and N). A representative volunteer's sample chromatogram for caffeine analysis is shown in Fig 4:12. The LC/MS/MS chromatograms of metoprolol analysis for blank plasma and plasma spiked with the internal standard did not show any interfering peaks (Appendix O and P). Volunteer's sample chromatogram showing the peaks for metoprolol, α-hydroxymetoprolol and 7-hydroxywarfarin is shown in Fig. 4:13.

The average retention times for paracetamol (IS), paraxanthine and caffeine were 4.9 min, 7.4 min and 15 min respectively. The total run time was 20 minutes. While the average retention times for α-hydroxymetoprolol, metoprolol and 7-hydroxywarfarin (IS) were 1.39 min, 1.41 min, and 5.89 min, respectively. The total run time for metoprolol analysis was 9.0 min.

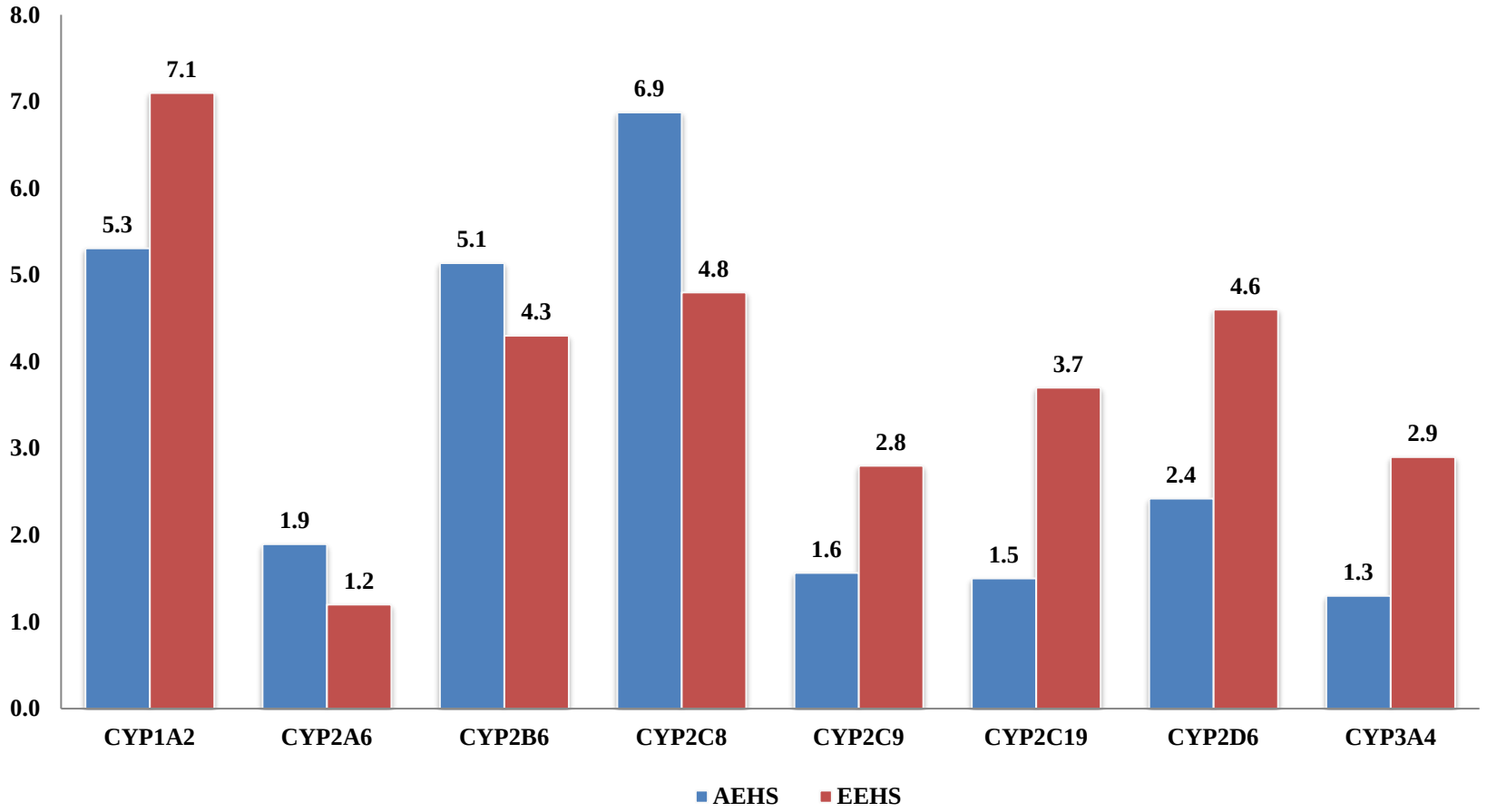


Fig 4.11: Fifty percent inhibitory concentration in litre per dose by the extracts of *Hibiscus sabdariffa in vitro*. For CYP2C19 and CYP3A4, average values of all the metabolites monitored is shown. AEHS – aqueous extract of *Hibiscus sabdariffa*, EEHS ethanolic extract of *Hibiscus sabdariffa*.

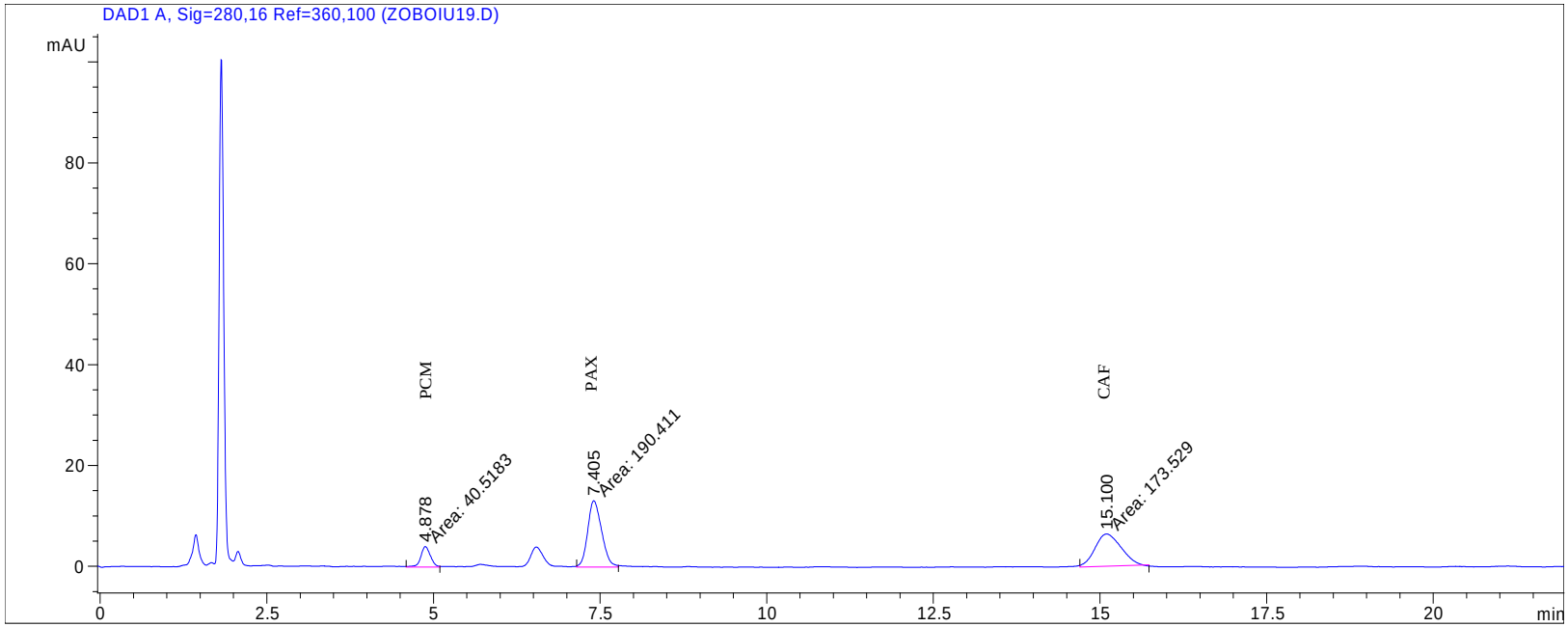


Fig 4.12: Chromatogram showing a representative sample. Caffeine (CAF), paraxanthine (PAX) and paracetamol (PCM, internal standard).

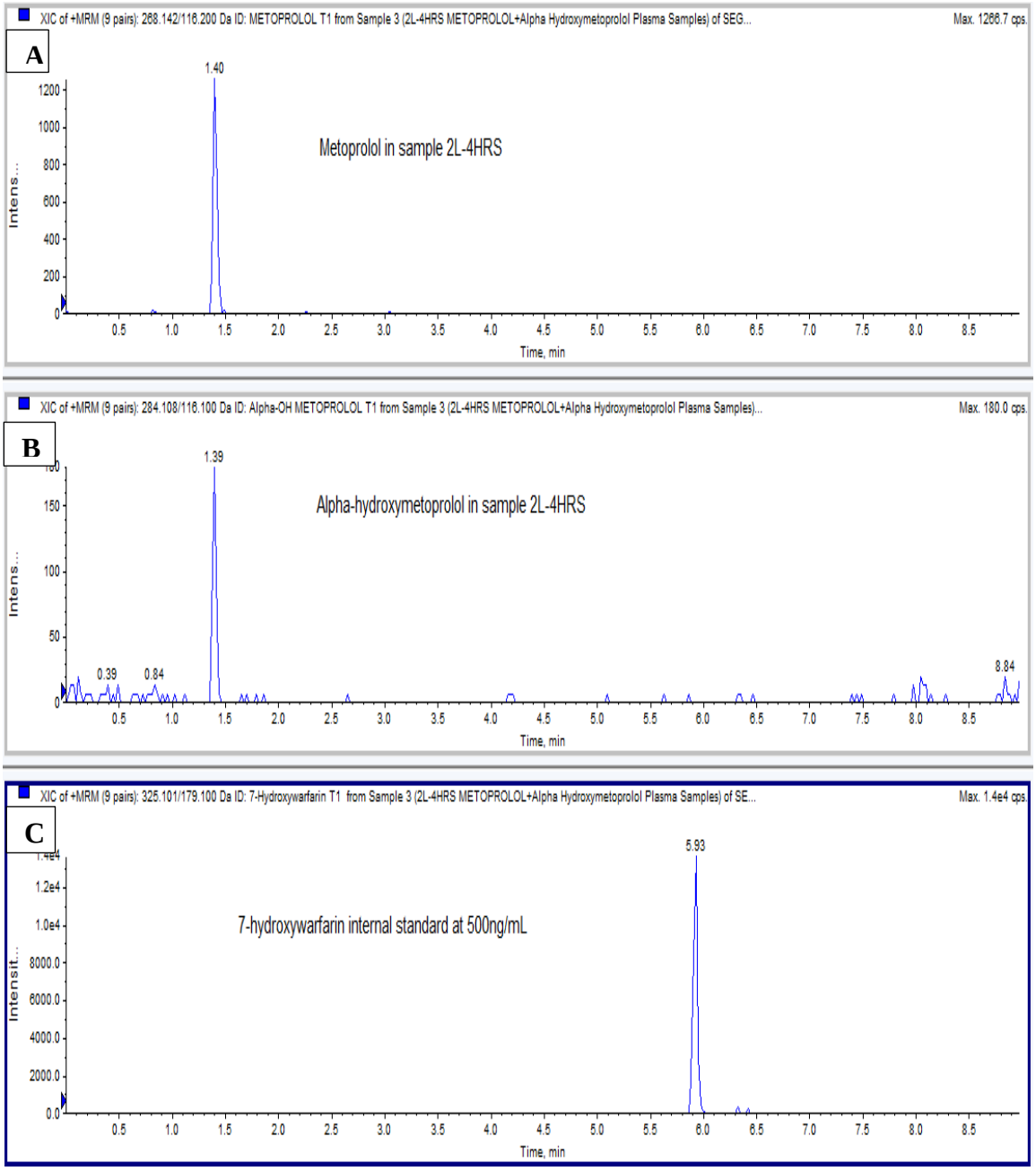


Fig 4.13: LC/MS/MS chromatogram for a volunteers' plasma sample (2L-4hrs) showing metoprolol (A), α-hydroxymetoprolol (B) and 7-hydroxywarfarin (C) peaks.

The lowest limit of quantification taken as the lowest calibration concentration with acceptable precision and accuracy for caffeine was 1 μg/mL and for paraxanthine it was 0.05 μg/mL. For metoprolol and α-hydroxymetoprolol the LLOQ was 10 ng/mL with 102.66% accuracy and 5.95% C.V precision. The validation parameters for the analysis of caffeine and metoprolol are within the acceptable values. Precision values for caffeine was less than 11% C.V, for paraxanthine it was < 7% C. V and for metoprolol and α-hydroxymetoprolol precision was <10% C.V. Accuracy for caffeine, paraxanthine and metoprolol were between 89-99%, 89-99% and 87-91%, respectively. Also the percentage recovery for caffeine and paraxanthine were 99-100% and 80-101%, respectively (see Appendix Q to S).

The plasma concentrations of caffeine with and without the concomitant intake of ABHS were 3.05 ± 0.16 μg/mL and 3.03 ± 0.19 μg/mL, respectively while the plasma concentrations of paraxanthine with and without the concomitant intake of *Hibiscus sabdariffa* beverage were 0.75 ± 1.84 μg/mL and 0.40 ± 0.13 μg/mL, respectively with no statistically significant differences. The phenotypic metabolic ratio (PMR) of caffeine with administration of caffeine and water was 0.16 ± 0.06 (range 0.10 to 0.35) and 0.31 ± 0.80 (range 0.11 to 3.94) when administered with ABHS. No significant difference in the ratios (p=0.348), as shown in Fig 4.14. When four outliers were removed there was still no statistically significant difference in the PMR of caffeine (p=0.858).

The concentration of metoprolol in plasma when administered with water was 8.89 ± 3.59 ng/mL and when administered with ABHS it was 13.50 ± 3.41 ng/mL while the concentrations of α-hydroxymetoprolol with and without coadministration with ABHS were 3.65 ± 2.83 ng/mL and 2.52 ± 1.48 ng/mL, respectively. The phenotypic metabolic ratio (PMR) of metoprolol with and without ABHS was 5.68 ± 2.14 and 4.24 ± 2.14, respectively. There was no statistically significant difference in these values (p=0.287). Figure 4.15 shows the single time point PMR for each volunteer.

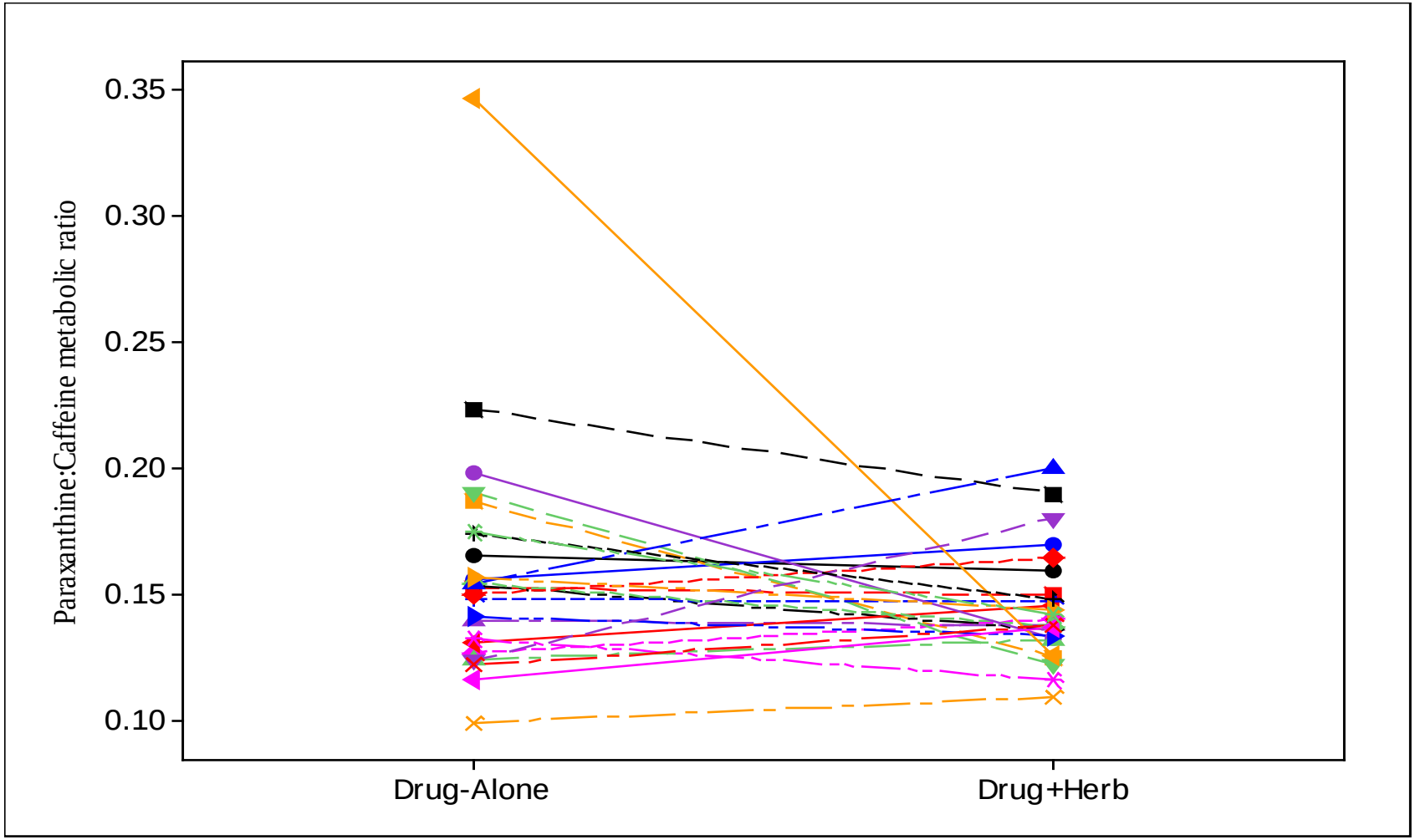


Fig 4.14: Single time point phenotypic metabolic ratio for caffeine when coadministered with aqueous beverage of *Hibiscus sabdariffa*

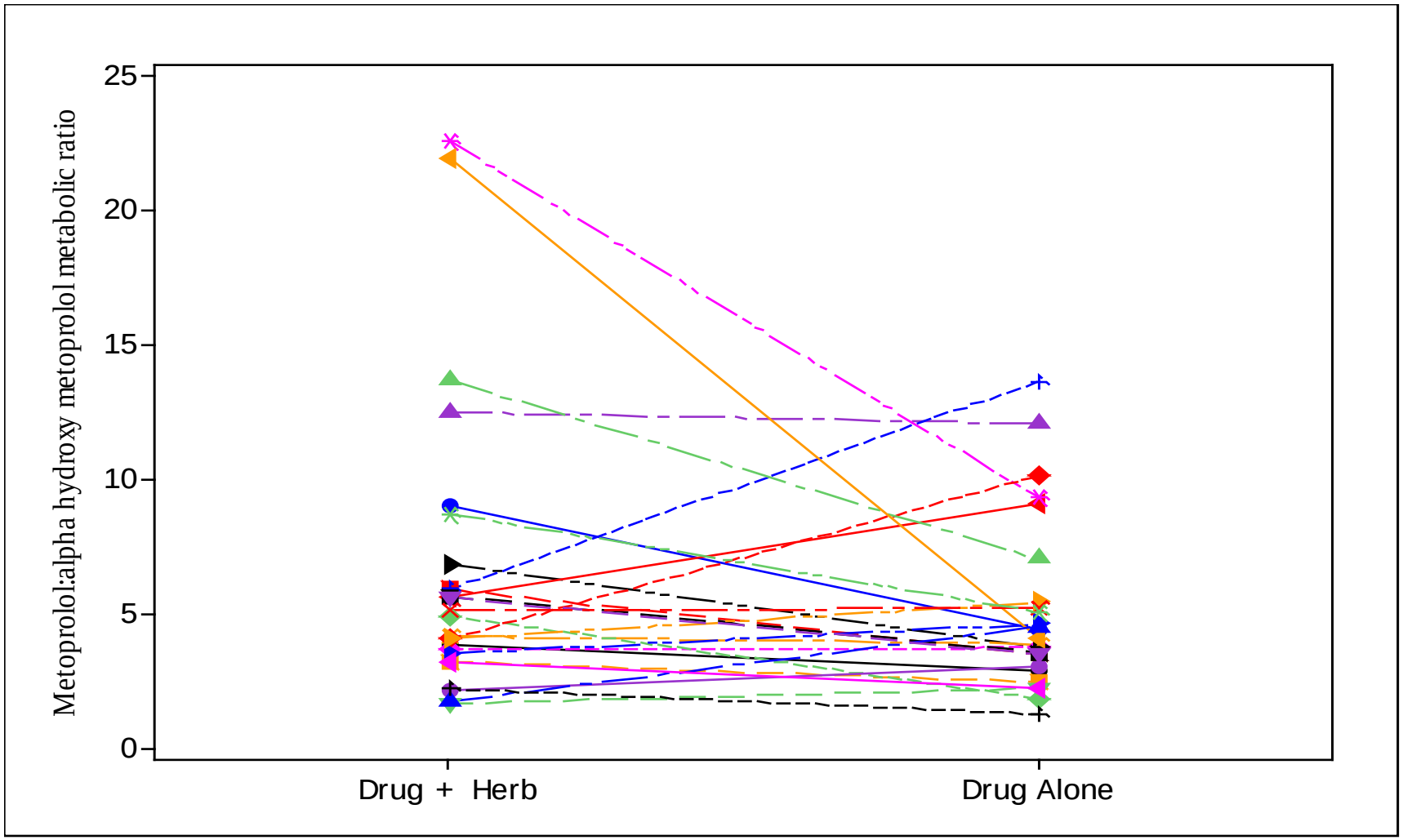


Fig4.15: **Single time point phenotypic metabolic ratio for metoprolol when coadministered with aqueous beverage of *Hibiscus sabdariffa.***

4.5 Effect of aqueous beverage of *Hibiscus sabdariffa* on the pharmacokinetic parameters of caffeine and simvastatin

The mean values of the laboratory tests for liver function test, kidney function test, lipid profile and other relevant laboratory indices for the volunteers in the analysis of caffeine and simvastatin were within normal range (see Appendix T).

The calibration curves for caffeine, paraxanthine and simvastatin were linear over the calibration concentration ranges of 0.05 to 15.0 μg/mL, 0.4 to 15 μg/mL and 0.01 to 10 μg/mL, respectively (see Appendix U to W). The analytes and the internal standard peaks were well resolved without interfering endogenous peaks. Figures 4.16 and 4.17 show the volunteers' plasma sample chromatogram for caffeine and simvastatin, respectively. Retention times for paraxanthine, etofylline (IS) and caffeine were 3.9 min, 4.9 min and 7.2 min, respectively. Total run time for the analyses of caffeine and paraxanthine was 10 minutes. The retention times for lovastatin (IS) and simvastatin were 3.3 min and 4.1 min, respectively, while the total run time for the analysis was 6 minutes. Precision of the method used for the analyses of caffeine, paraxanthine and simvastatin was less than 15% coefficient of variation for both intra-day and inter-day runs. Likewise the accuracy of the method for all the quality control samples (LQC, MQC and HQC) and LLOQ were within 85 – 115% and 80 – 120% of the nominal values, respectively. The detail of the method validation is shown in Appendix X. Recoveries of analyte were more than 75%.for caffeine and more than 65% for simvastatin.

Concurrent administration of ABHS with caffeine, a CYP1A2 substrate, decreased the rate of absorption (K_a) of caffeine significantly by 30.0% and the elimination rate constant (K_e) was increased by 23.0% (Table 4.6). The 30% decrease in the rate of absorption (K_a) of caffeine when coadministered with ABHS did not change the extent of absorption of caffeine. Area under the plasma concentration-time curve from 0 to 24 hours ($AUC_{0\text{-}24h}$) remained the same when caffeine was coadministered with water ($AUC_{0\text{-}24h}$ = 33.51 μgml-1hr) and with ABHS, $AUC_{0\text{-}24h}$ = 33.29 $\mu gml^{-1}h$, as shown in Table 4.6 and Fig 4.18.

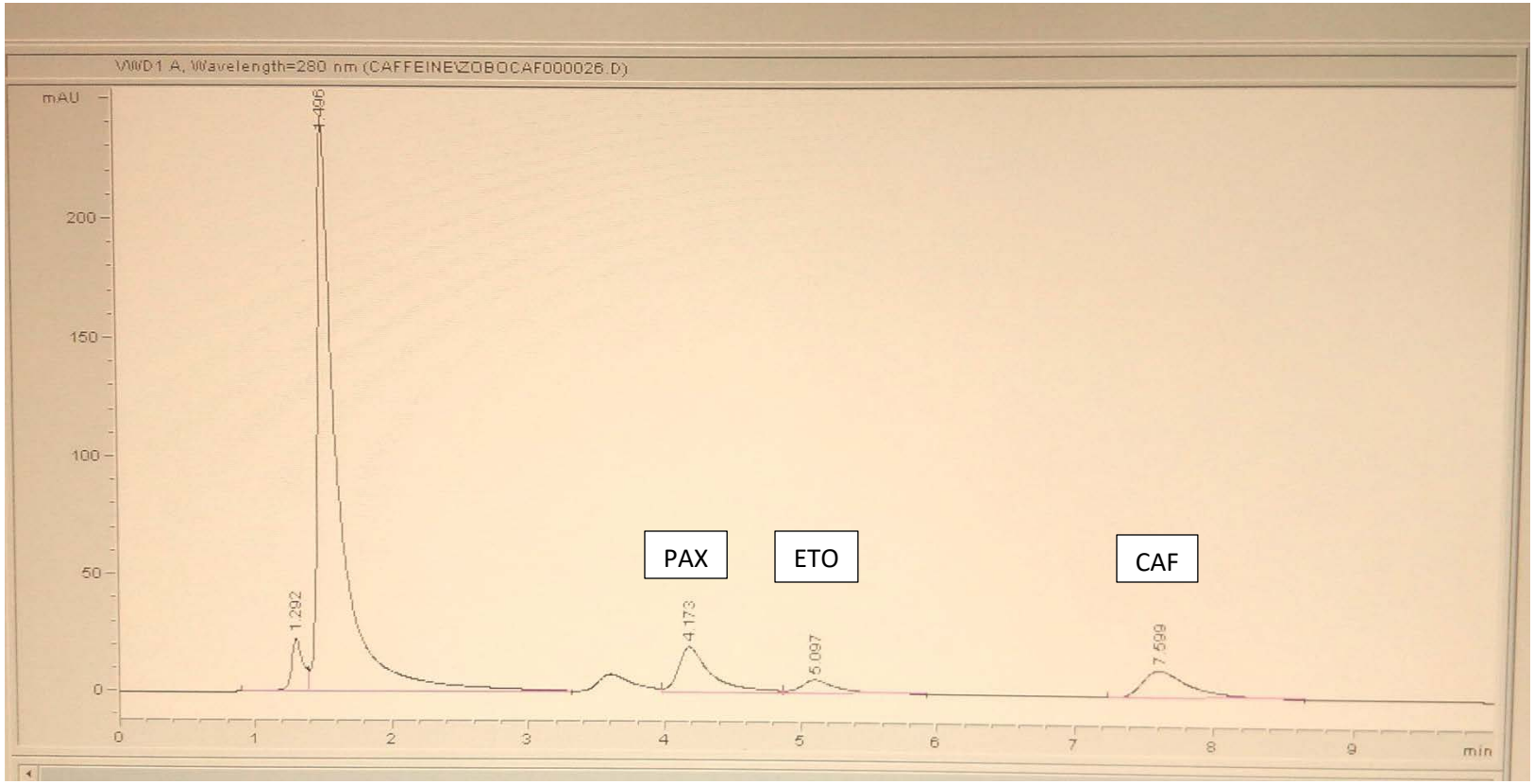


Fig 4:16: **A typical HPLC chromatogram of the volunteers in the analysis of caffeine showing paraxanthine (PAX), etofylline (ETO), and caffeine (CAF) at 4.17 min, 5.09 min, and 7.59 min, respectively**

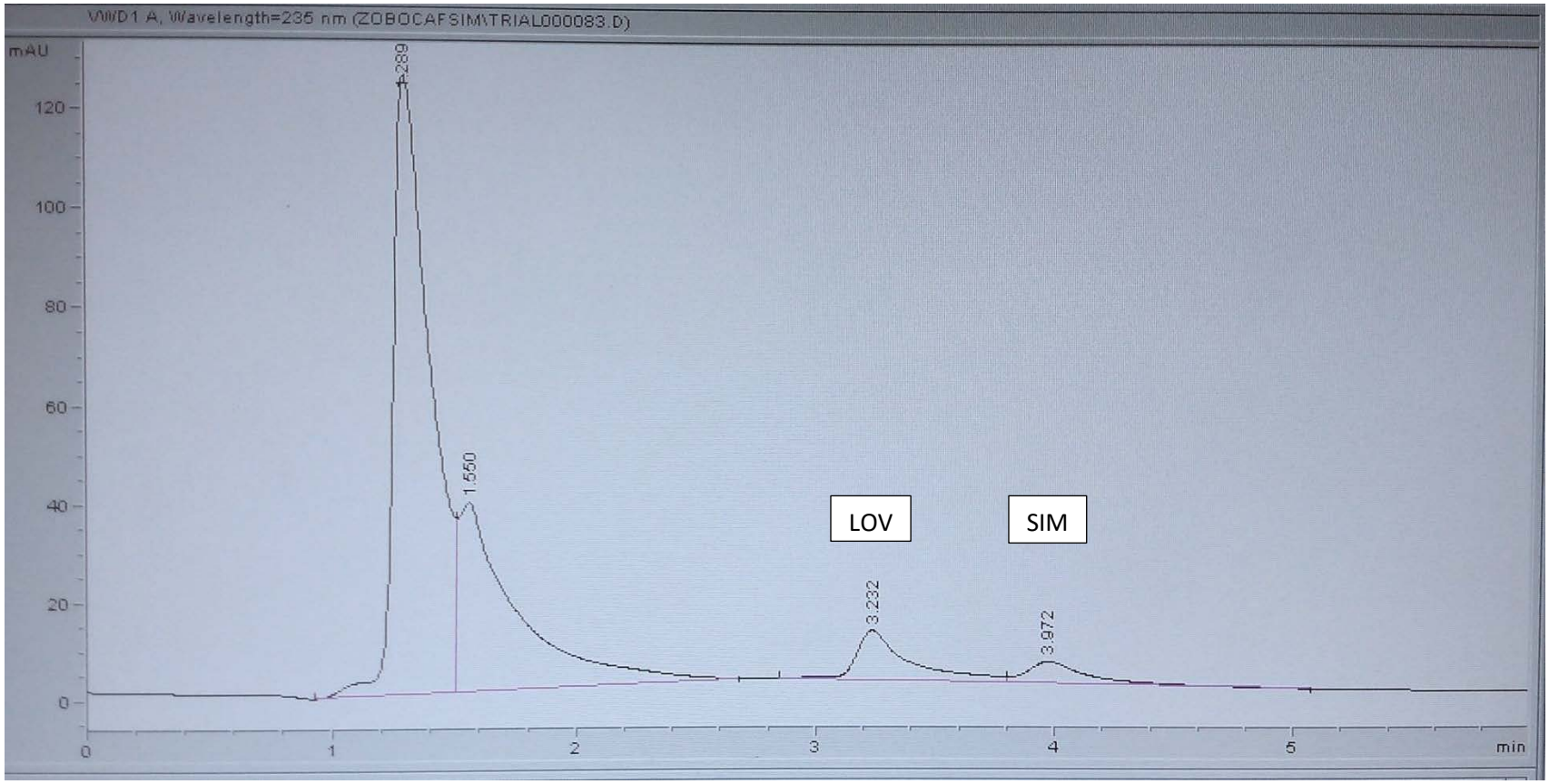


Fig 4:17: **A typical HPLC chromatogram of the volunteers in the analysis of simvastatin showing lovastatin (LOV) and simvastatin (SIM) at 3.23 min and 3.97 min.**

Table 4.6: Pharmacokinetic parameter of caffeine when coadministered with water and aqueous beverage of *Hibiscus sabdariffa*

Pharmacokinetic parameter	Caffeine + water Mean ± SD	Caffeine + aqueous beverage of *Hibiscus sabdariffa* Mean ± SD	p-value[a]	% Fold change
K_a (h^{-1})	3.80 ± 2.08	2.66 ± 0.98	0.958	-30.0%
K_e (h^{-1})	0.13 ± 0.04	0.16 ± 0.03	0.100	21.0%
$t_{1/2}$ K_a (h^{-})	0.18 ± 0.44	0.26 ± 0.16	0.420	43.0%
$t_{1/2}$ K_e (hr)	5.24 ± 2.18	4.37 ± 0.75	0.825	-15.0%
V/F (L)	43.15 ± 18.46	37.01 ± 4.14	0.443	-14.0%
CL/F (Lh^{-1})	5.71 ± 0.87	5.86 ± 2.36	0.769	3.0%
T_{max} (h)	0.91 ± 0.85	1.13 ± 0.55	0.949	23.0%
C_{max} (μgmL^{-1})	4.11 ± 1.39	4.52 ± 0.59	0.535	10.0%
AUC_{0-24} ($\mu gmL^{-1}h$)	33.51 ± 3.82	33.29 ± 9.58	0.749	-1.0%
$AUC_{0-\infty}$ ($\mu gmL^{-1}h$)	35.03 ± 3.76	34.10 ± 10.07	0.158	-3.0%
AUMC ($\mu gmL^{-1}h^2$)	273.94 ± 51.81	227.99 ± 95.01	0.968	-17.0%
MRT (h)	7.82 ± 2.79	6.69 ± 1.19	0.774	-14.0%

Mean residence time (MRT), Area under the first moment curve (AUMC), SD – standard deviation, [a]Paired sample T-test

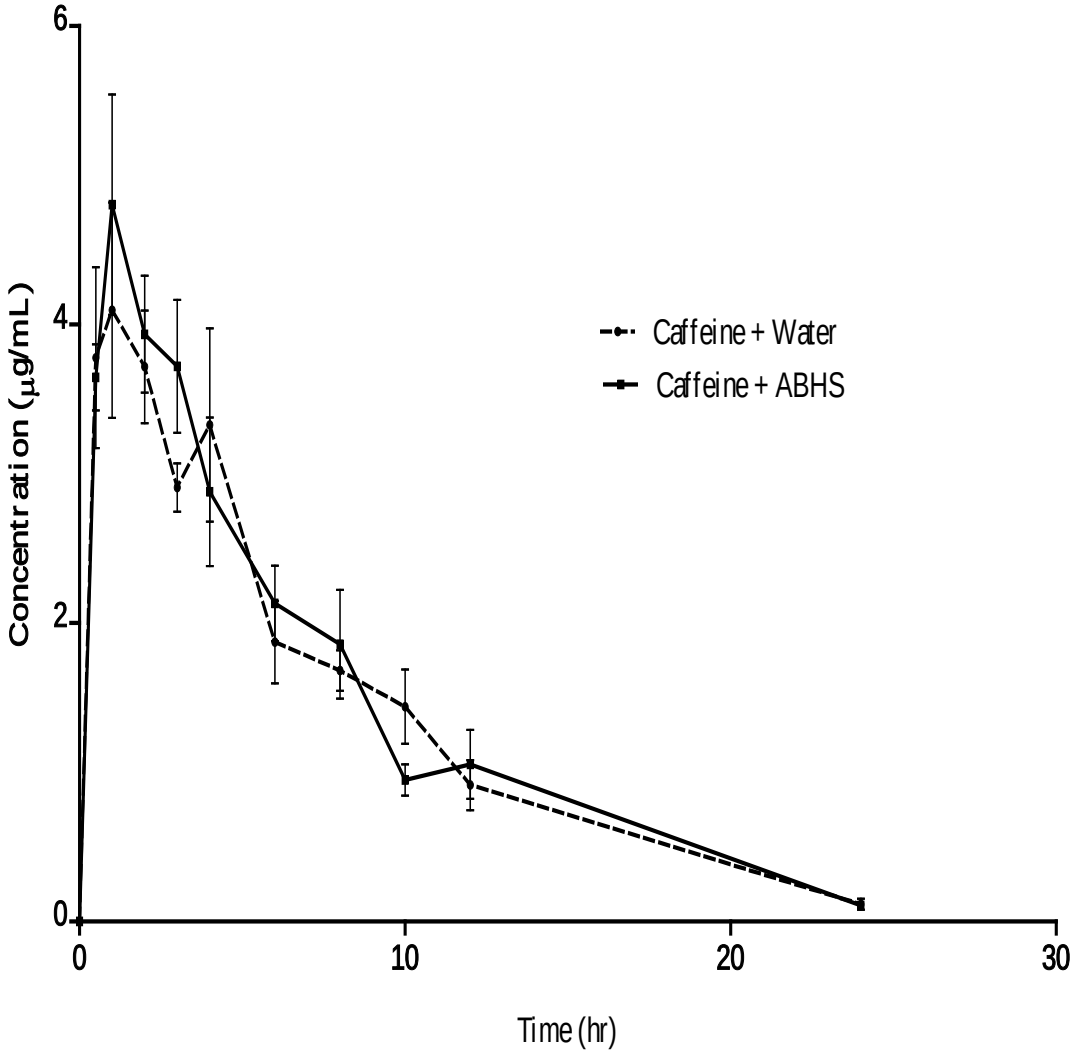


Fig 4.18: Plasma concentration-time curve of caffeine when coadministered with water and aqueous beverage of *Hibiscus sabdariffa* (ABHS)

Aqueous beverage of *Hibiscus sabdariffa* increased caffeine C_{max} by 10.0%, from 4.1 μg/mL to 4.5 μg/mL,.while apparent clearance of caffeine (CL/F) remained largely unchanged. The apparent volume of distribution (V/F) was slightly decreased from 43 L to 37 L (Table 4.6). Figure 4.19 shows the marginal difference in the ratio of AUC of drug to metabolite when caffeine was administered with water or aqueous beverage of *Hibiscus sabdariffa.*

The absorption rate constant (K_a) and elimination rate constant (K_e) of simvastatin were reduced by 18.0% and 28.0%, respectively by aqueous beverage of *Hibiscus sabdariffa* when coadministered with the drug. These reductions resulted in 22.0% and 37.0% increases in absorption rate half-life ($t_{1/2}K_a$) and elimination rate half-life ($t_{1/2}K_e$), respectively (Table 4.7)

Apparent clearance of simvastatin (CL/F) was reduced by 52.0% with ABHS while the time taken to reach the maximum plasma concentration (T_{max}) was increased from 2.9 hrs to 3.6 hrs. Peak plasma concentration (C_{max}) and area under the plasma concentration-time curve ($AUC_{0\text{-}24hrs}$) were decreased by 18.0% and 31.0%, respectively by ABHS. Other changes in the pharmacokinetic parameter of simvastatin with concurrent administration of ABHS are shown in Table 4.7. The plasma concentration-time curve for simvastatin taken with water or aqueous beverage of *Hibiscus sabdariffa* is shown in Fig 4.20.

4.6 Interactions between aqueous extract of *Hibiscus sabdariffa* and simvastatin

In the study of interactions of aqueous extract of *Hibiscus sabdariffa* with simvastatin, average percentage weight gain of rats in the treatment groups ranged from 13.05% to 29.85%. These were not statistically significantly different from the control groups. The ranking of individual effect for total cholesterol, T_c, was N>P>>D, for triglyceride (TG) and low density lipoprotein cholesterol (LDL_c) it was P>>N>>D and P>>D>N for high density lipoprotein cholesterol, HDL_c, (Table 4.8).

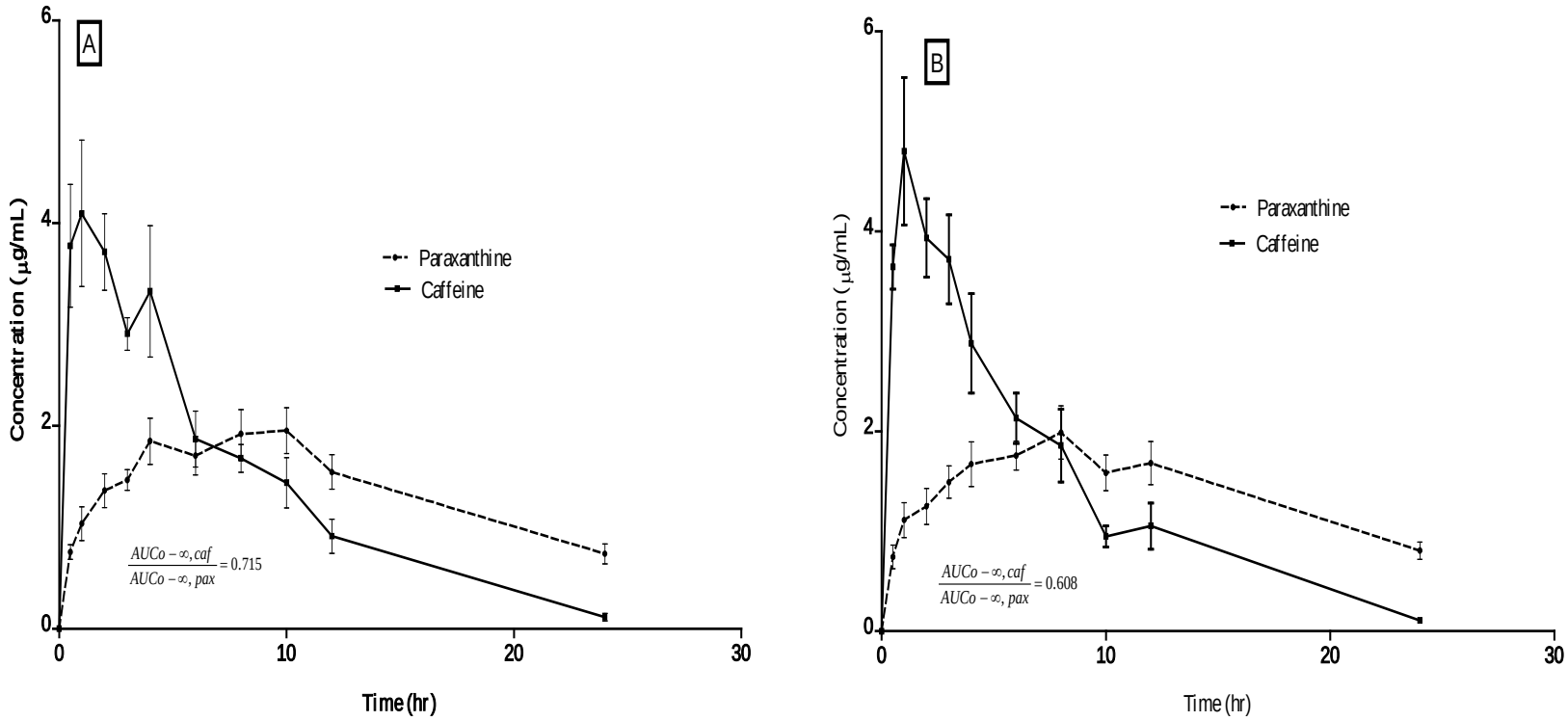


Fig 4.19: Change in the $AUC_{0-\infty}$,caffeine/ $AUC_{0-\infty}$,paraxanthine ratio when caffeine was administered with water (A) and aqueous beverage of *Hibiscus sabdariffa* (B).

Table 4.7: Pharmacokinetic parameter of simvastatin when coadministered with aqueous beverage of *Hibiscus sabdariffa*

Pharmacokinetic parameter	Simvastatin + water (Mean ± SD)	Simvastatin + aqueous beverage of *Hibiscus sabdariffa* (Mean ± SD)	p-value	% Fold change
K_a (h^{-1})	1.16 ± 1.10	0.95 ± 0.65	0.291	-18.0%
K_e (h^{-1})	0.05 ± 0.13	0.03 ± 0.14	0.253	-28.0%
$t_{1/2}$ K_a (h^{-})	0.60 ± 0.68	0.73 ± 0.48	0.790	22.0%
$t_{1/2}$ K_e (hr)	14.86 ± 6.06	20.30 ± 15.37	0.505	37.0%
V/F (L)	23.50 ± 12.93	26.91 ± 5.20	0.511	15.0%
CL/F (Lh^{-1})	1.91 ± 0.90	0.92 ± 0.77	0.395	-52.0%
T_{max} (h)	2.89 ± 5.42	3.62 ± 2.90	0.173	25.0%
C_{max} (μgmL^{-1})	1.49 ± 0.42	1.31 ± 0.11	0.133	-12.0%
AUC_{0-24} ($\mu gmL^{-1}h$)	34.08 ± 7.33	23.64 ± 6.55	0.516	-31.0%
$AUC_{0-\infty}$ ($\mu gmL^{-1}h$)	36.50 ± 34.23	43.53 ± 29.39	0.349	19.0%
AUMC ($\mu gmL^{-1}h^2$)	814.32 ± 85.38	1320.06 ± 234.59	0.519	62.0%
MRT (h)	22.30 ± 22.303	30.33 ± 21.55	0.663	36.0%

Mean residence time (MRT), Area under the first moment curve (AUMC), SD – standard deviation, [a]Paired sample T-test.

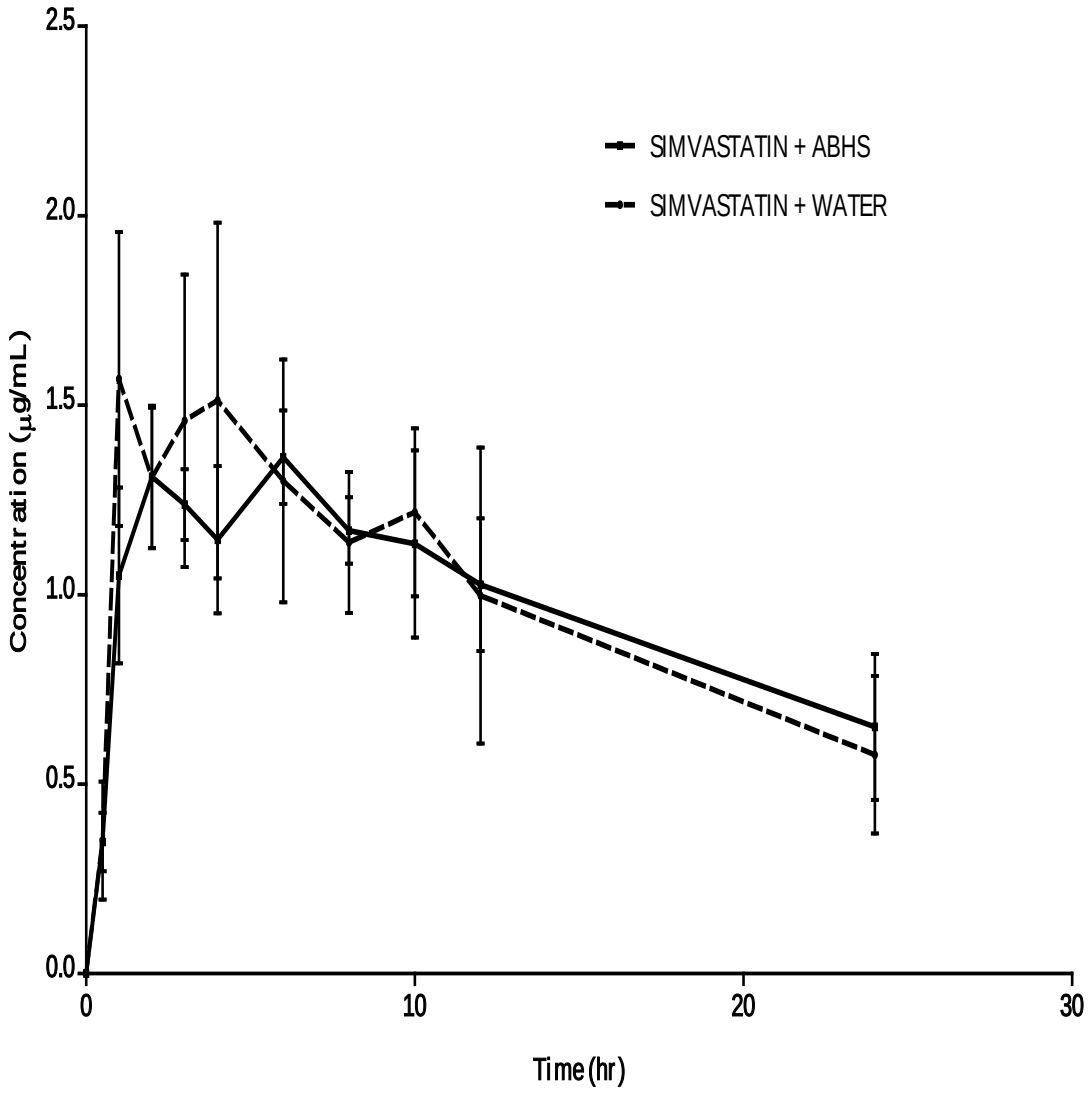


Fig 4.20: Plasma concentration time profile for simvastatin when coadministered with water and aqueous beverage of *Hibiscus sabdariffa*

Period of treatment had statistically significant effect on TG (p=0.018), HDL_c (p=0.017) and LDL_c (p=0.002) as shown in Table 4.8. Significant interaction was observed between N-D for T_c (p=0.001) as shown in Table 4.9, such that for the herb, higher dose was more effective in reducing T_c [F(1, 32) = 14.749, p=0.001] but for the drug, increasing the dose had no significant effect on the extent of reduction in T_c [F(1, 32) = 7.314, p=0.110]. For N-P, the interactions were additive for TG and of opposing effect for HDL_c and LDL_c (Table 4.9). The pattern of N-P interaction (Fig 4.21 to 4.25) was such that for the drug, simvastatin, extending the period of treatment from two to four week gave a higher reduction in TG (p=0.001), moderate decrease in HDL_c (p=0.001) and lesser reduction in LDL_c (p=0.001) while there was no significant change in TG, HDL_c and LDL_c with the herb, AEHS, (p>0.05).

However, concurrent administration of AEHS and simvastatin in various dose combinations did not show statistically significant changes in the lipid profile when compared to AEHS administered alone (p>0.05) as shown in Table 4.10. But, combinations of low-dose-AEHS + low-dose-simvastatin, low-dose-AEHS + high-dose-simvastatin, and high-dose-AEHS + high-dose-simvastatin, administered for two weeks gave 38.26% (p=0.026), 36.96% (p=0.040), and 44.13% (p=0.003) statistically significant reductions in T_c level compared to 22.41% reduction by low-dose-simvastatin (10 mg/kg). Also, there was 57.43% and 47.26% reduction in TG when combinations of low-dose-AEHS + low-dose-simvastatin and high-dose-AEHS + low-dose-simvastatin were administered to the rats respectively for two weeks. These values were significantly different from 8.10% reduction in TG level observed with low-dose-simvastatin (p<0.05) as shown in Table 4.10. The effect of coadministration of low-dose-AEHS and low-dose-simvastatin on LDL_c was not significantly different from when they were administered individually but combination of high-dose-AEHS + high-dose-simvastatin caused 80.56% reduction in LDL_c compared to 47.81% and 49.54% reduction in LDL_c level with low-dose-AEHS and low-dose-simvastatin, respectively (p<0.05) as shown in Table 4.10.

Table 4.8: Individual coefficients for factors combinations using percentage change in lipid profile for individual administration of herb (AEHS) and drug (simvastatin)

		INDIVIDUAL EFFECTS			
		Tc (%↓)	**TG (%↓)**	**HDLc (%↑)**	**LDLc (%↓)**
N	Individual coefficient	-8.650	-15.698	-3.755	-16.675
	P-value	0.031*	0.104	0.730	0.010*
D	Individual coefficient	-1.360	-6.343	6.865	-3.485
	P-value	0.833	0.504	0.529	0.572
P	Individual coefficient	-6.160	23.413	-27.235	-20.815
	P-value	0.172	0.018*	0.017*	0.002**
Ranking		N>P>D	P>N>D	P>D>N	P>N>D

**P<0.05, **P<0.01.* AEHS – aqueous extract of *H. sabdariffa*, N – nature of agent (herb or drug), D – dose, and P – treatment period

Table 4.9: Interaction coefficients for factor combinations using percentage change in lipid profile for individual administration of herb (AEHS) and drug (simvastatin)

		INTERACTION EFFECTS			
		Tc (%↓)	**TG (%↓)**	**HDLc (%↑)**	**LDLc (%↓)**
N - D	Interaction coefficient	-15.805	-10.283	-9.470	-9.515
	P-value	0.001**	0.281	0.387	0.128
N - P	Interaction coefficient	4.105	22.973	-31.120	-17.005
	P-value	0.265	0.020*	0.007**	0.009**
D - P	Interaction coefficient	-11.005	-0.042	11.900	-18.265
	P-value	0.008**	0.997	0.278	0.005**
Ranking		N-D>D-P>N-P	N-P>N-D>D-P	N-P>D-P>N-D	D-P>N-P>N-D

**P<0.05, **P<0.01.* AEHS – aqueous extract of *H. sabdariffa*, N – nature of agent (herb or drug), D – dose, and P – treatment period

Table 4.10: Percentage change in lipid profile with concomitant and individual administration of aqueous extract of *Hibiscus sabdariffa* and simvastatin in hyperlipidemia-induced rats

Lipid profile/ Treatment period	Controls		Treatment groups							
			Individual treatment				Concomitant treatment			
	Vehicle control	Negative control	AEHS 250 mg/kg	AEHS 500 mg/kg)	Sim 10 mg/kg	Sim 20 mg/kg	AEHS 250 mg/kg + Sim 10 mg/kg	AEHS 250 mg/kg + Sim 20 mg/kg	AEHS 500 mg/kg + Sim 10 mg/kg	AEHS 500 mg/kg + Sim 20 mg/kg
TOTAL CHOLESTEROL (Percentage decrease)										
2 weeks	11.80 ± 6.81	9.72 ± 15.51	31.67 ± 11.89	46.81 ± 4.92	22.41 ± 7.93	28.57 ± 11.24	38.26 ± 11.91[c]	36.96 ± 9.72[c]	26.06 ± 11.06[b]	44.13 ± 12.94[c]
4 weeks	15.70 ± 23.80*	15.51 ± 10.94	22.11 ± 6.78	35.86 ± 7.25	43.67 ± 16.41	8.97 ± 24.35	23.86 ± 16.06[c]	37.24 ± 2.91[d]	35.60 ± 13.12[d]	27.85 ± 15.53
TRIGLYCERIDE (Percentage decrease)										
2 weeks	10.84 ± 48.42*	15.99 ± 37.30	40.29 ± 14.01	40.47 ± 8.37	8.10 ± 58.59	4.70 ± 38.02*	57.43 ± 28.15[c,d]	14.24 ± 53.93	47.26 ± 25.34[d]	23.05 ± 38.65
4 weeks	3.14 ± 51.76*	15.42 ± 28.76	36.97 ± 33.03	44.66 ± 25.54	58.32 ± 9.15	37.86 ± 8.81	26.58 ± 10.99	6.70 ± 57.80*[a,b,c,d]	24.20 ± 40.18 [a,b,c,d]	9.11 ± 27.95[c]
HIGH DENSITY LIPOPROTEIN CHOLESTEROL (Percentage increase)										
2 weeks	40.84 ± 49.64	2.64 ± 9.00	54.21 ± 33.95	61.66 ± 25.20	94.06 ± 21.03	76.54 ± 40.16	53.10 ± 19.69	62.21 ± 44.54	83.74 ± 26.42	51.72 ± 32.56[c,d]
4 weeks	31.79 ± 4.92	0.09 ± 16.45	49.21 ± 31.33	74.43 ± 44.38	20.79 ± 30.70	33.10 ± 39.71	3.98 ± 9.22[a,b]	23.36 ± 32.90[b]	45.20 ± 37.86	17.84 ± 21.71
LOW DENSITY LIPOPROTEIN CHOLESTEROL(Percentage decrease)										
2 weeks	37.17 ± 25.68	6.08 ± 44.59	47.81 ± 15.08	63.99 ± 4.75	49.54 ± 6.70	62.92 ± 15.14	55.66 ± 17.54	56.93 ± 6.21	39.41 ± 10.21	80.56 ± 8.89[a,c]
4 weeks	27.96 ± 54.86	102.92 ± 99.39	54.15 ± 17.84	50.03 ± 5.33	38.10 ± 16.63	1.28 ± 39.89*	43.80 ± 17.86	56.1 ± 8.01[d]	56.10 ± 8.01[d]	18.02 ± 18.20* [a,b,c,d]

[a]p<0.05 compared to *AEHS 250 mg/kg*, *[b]p<0.05* compared to *AEHS 500 mg/kg*, *[c]p<0.05* compared to *Simvastatin 10 mg/kg and [d]p<0.05* compared to *Simvastatin 20 mg/k. *percentage increase for T_c, TG and LDL_c but percentage decrease for HDL_c*..AEHS – aqueous extract of *Hibiscus sabdariffa*

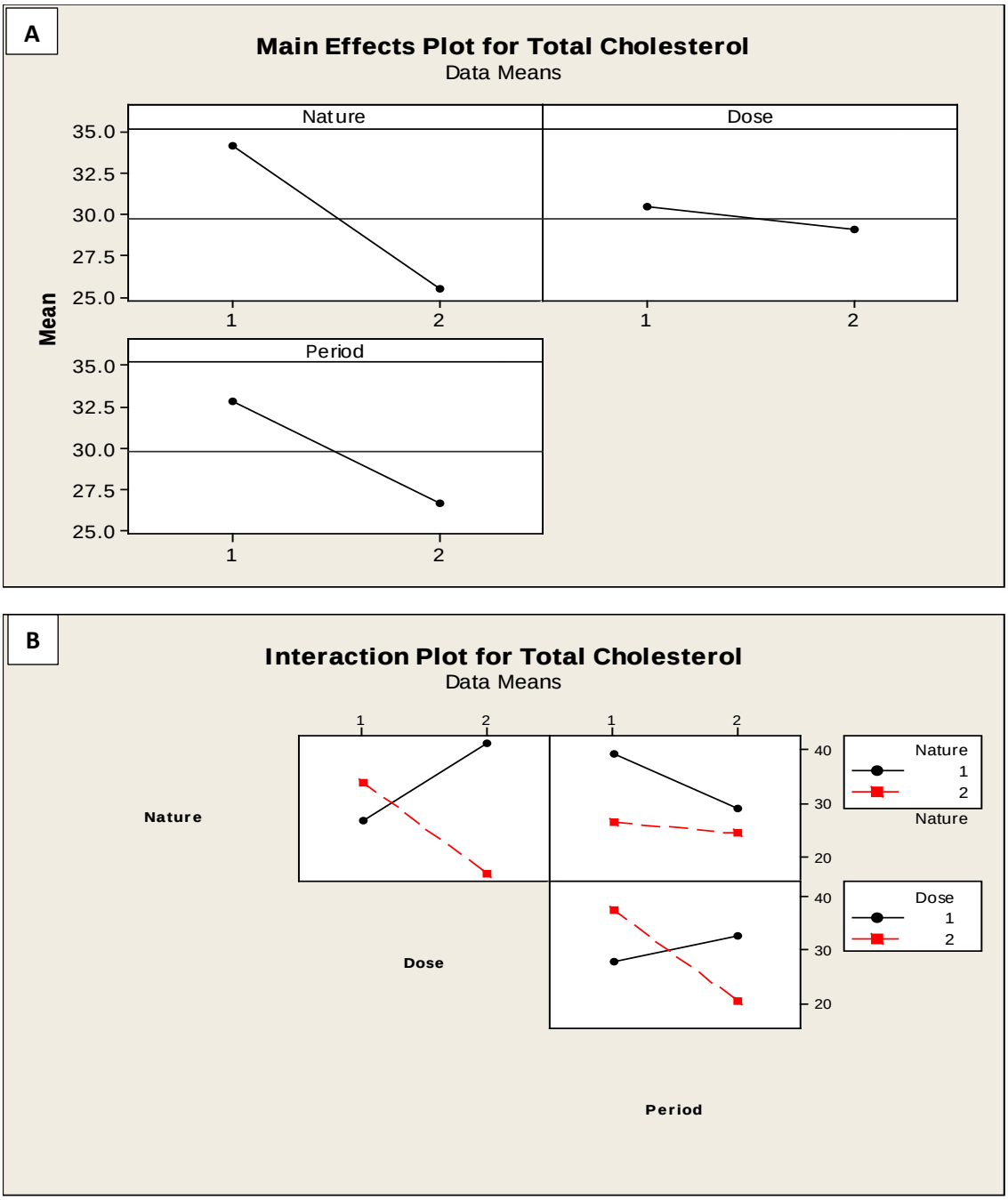


Fig 4.21: P**ercentage decrease in total cholesterol level, (A) when a factor (Nature, Dose, and treatment Period) is changed from low to high level; (B) when two factors (N-D, N-P, and D-P) interacts. Number 1 and 2 represent low and high level of each factor, respectively.**

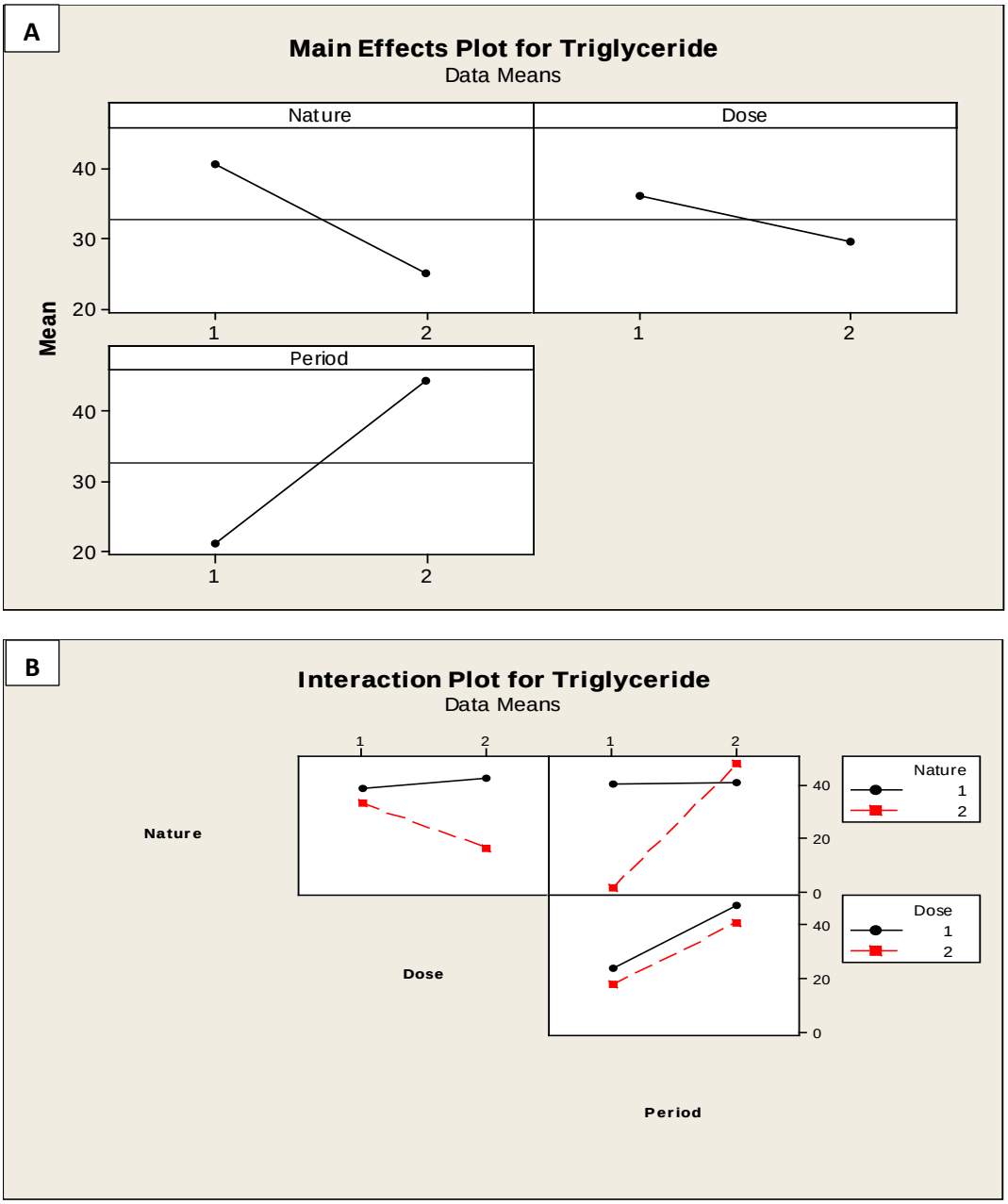


Fig 4.22:: Percentage decrease in triglyceride level, (A) when a factor (Nature, Dose, and treatment Period) is changed from low to high level; (B) when two factors (N-D, N-P, and D-P) interacts. Number 1 and 2 represent low and high level of each factor, respectively.

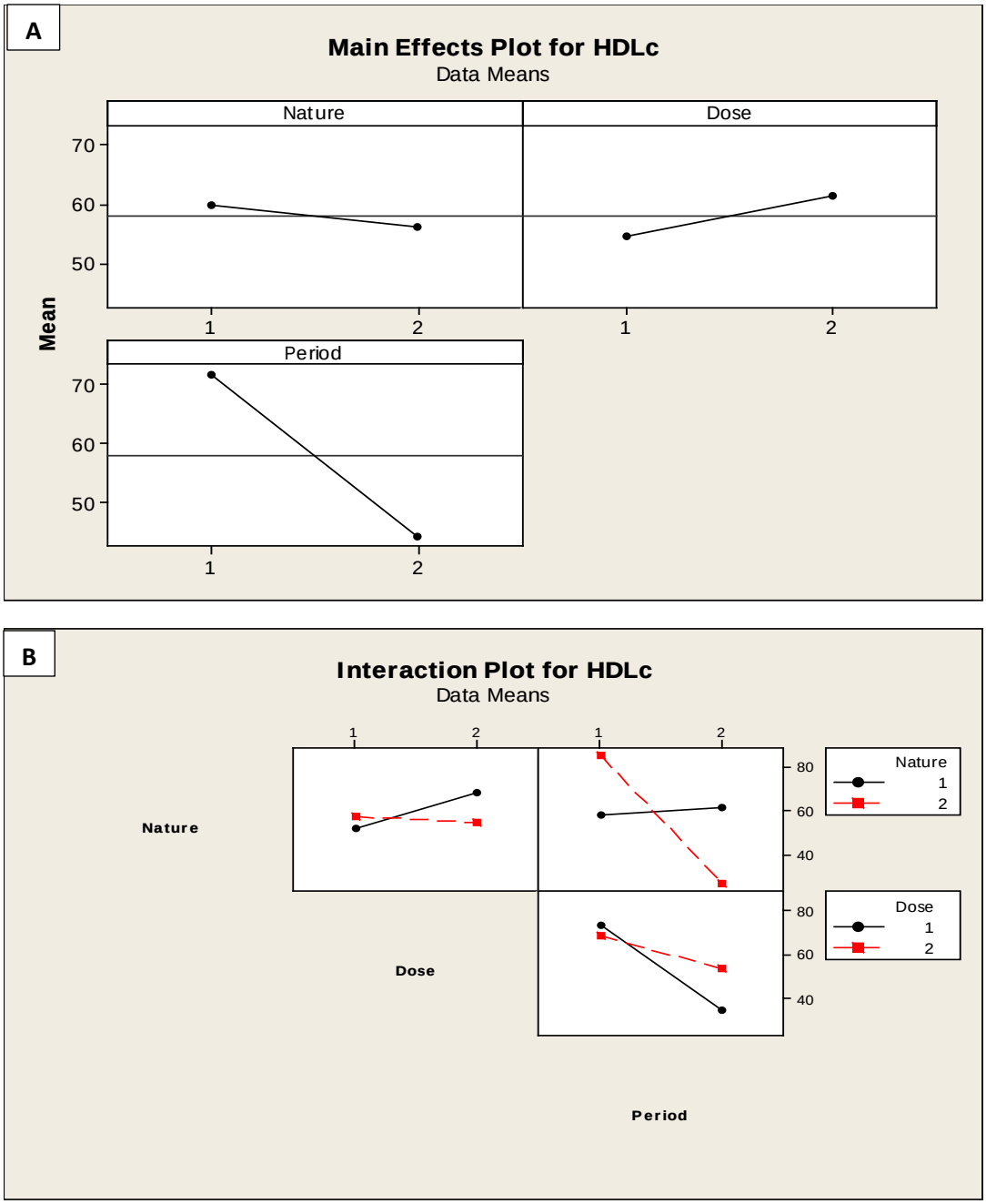


Fig 4.23:: Percentage increase in high density lipoprotein cholesterol level, (A) when a factor (Nature, Dose, and treatment Period) is changed from low to high level; (B) when two factors (N-D, N-P, and D-P) interacts. Number 1 and 2 represent low and high level of each factor, respectively.

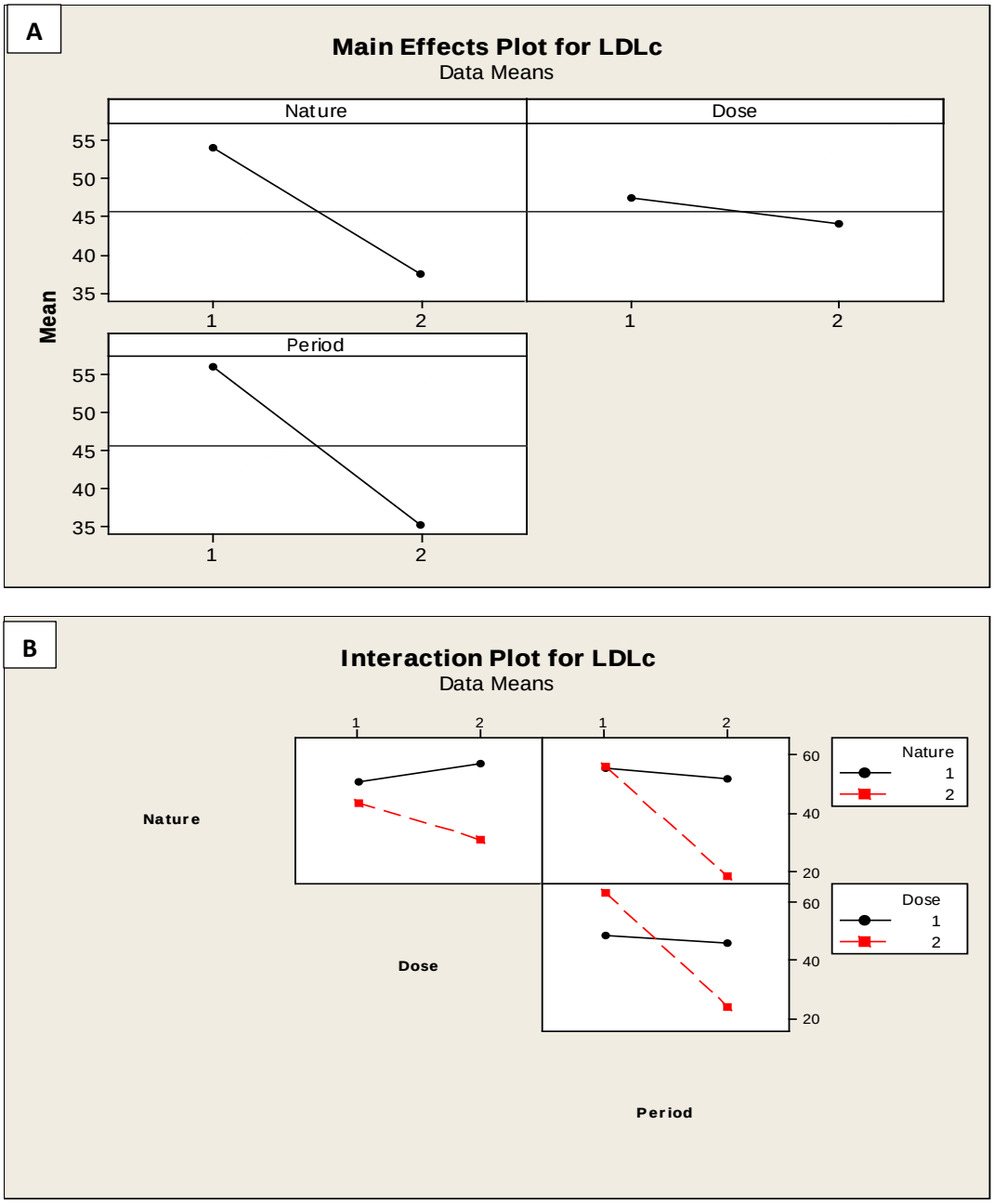


Fig 4.24: P**ercentage decrease in low density lipoprotein cholesterol level, (A) when a factor (Nature, Dose, and treatment Period) is changed from low to high level; (B) when two factors (N-D, N-P, and D-P) interacts. Number 1 and 2 represent low and high level of each factor, respectively.**

Interaction between AEHS and simvastatin (Z-S) when administered concomitantly was significant for HDL_c ($p<0.05$) and LDL_c ($p<0.01$). These interactions were of opposing effects (Fig 4.25 to 4.28). For low-dose-simvastatin, increasing the dose of AEHS in the combination led to a significant increase in HDL_c [$F(1, 32) = 4.896$, $p=0.034$], but for high-dose-simvastatin, an increase in the dose of AEHS in the combination caused a slight decrease in HDL_c (Fig 4.27). In the case of LDL_c, an increase in the dose of AEHS combined with low-dose-simvastatin did not cause a significant decrease in LDL_c ($p=0.236$) as shown in Fig 4.28, but when the dose of AEHS was increased in combination with high-dose-simvastatin, there was a significant smaller reduction in LDL_c ($p=0.0001$).

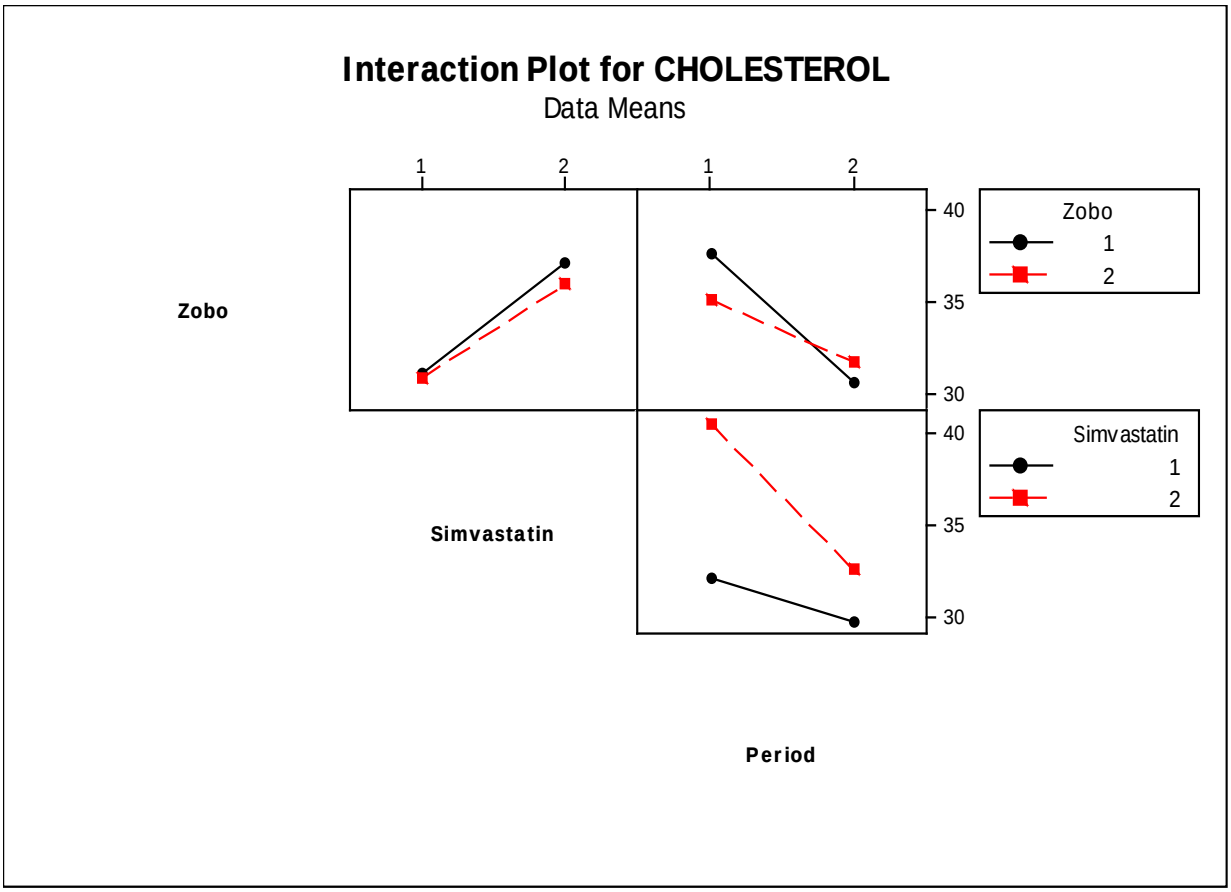


Fig 4.25: P**ercentage decrease in total cholesterol level, resulting from interactions between aqueous extract of *Hibiscus sabdariffa* (*Zobo*) and simvastatin, simvastatin and treatment period, and *Zobo* and treatment period. Number 1 and 2 represent low and high level of each factor, respectively.**

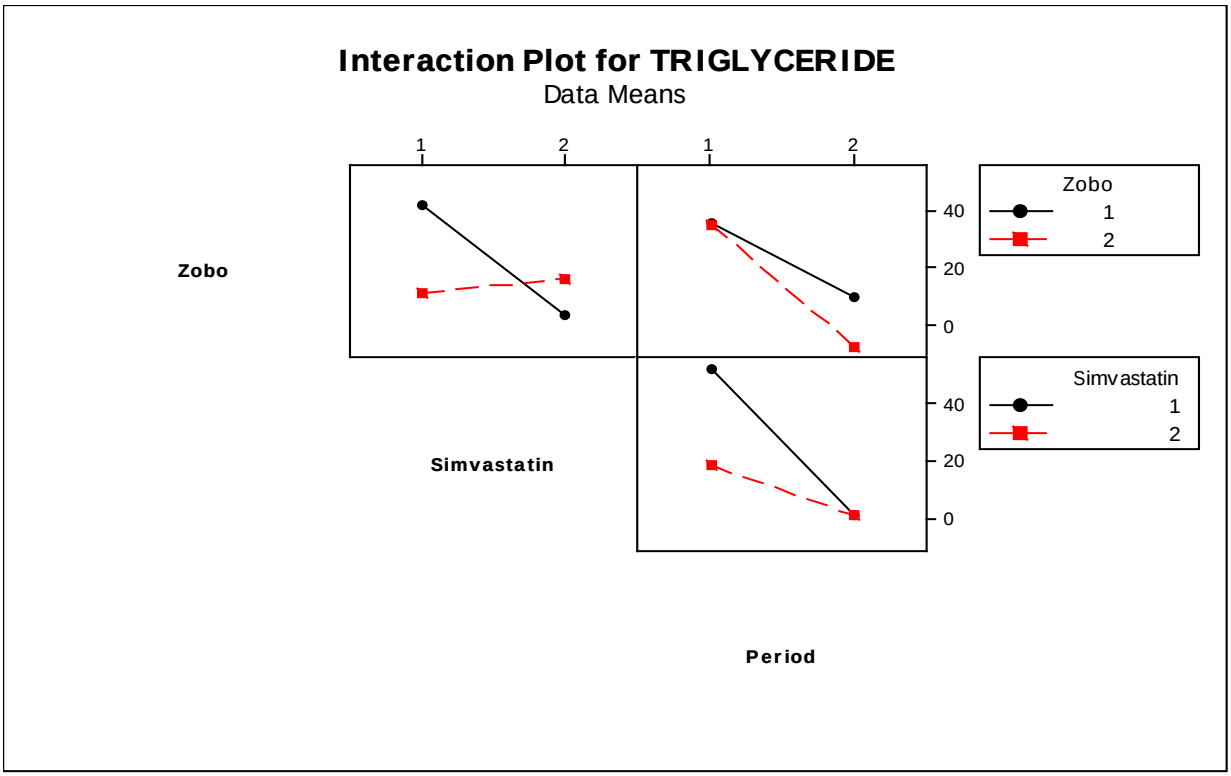


Fig 4.26: P**ercentage decrease in triglyceride level, resulting from interactions between aqueous extract of *Hibiscus sabdariffa* (*Zobo*) and simvastatin, simvastatin and treatment period, and *Zobo* and treatment period. Number 1 and 2 represent low and high level of each factor, respectively**

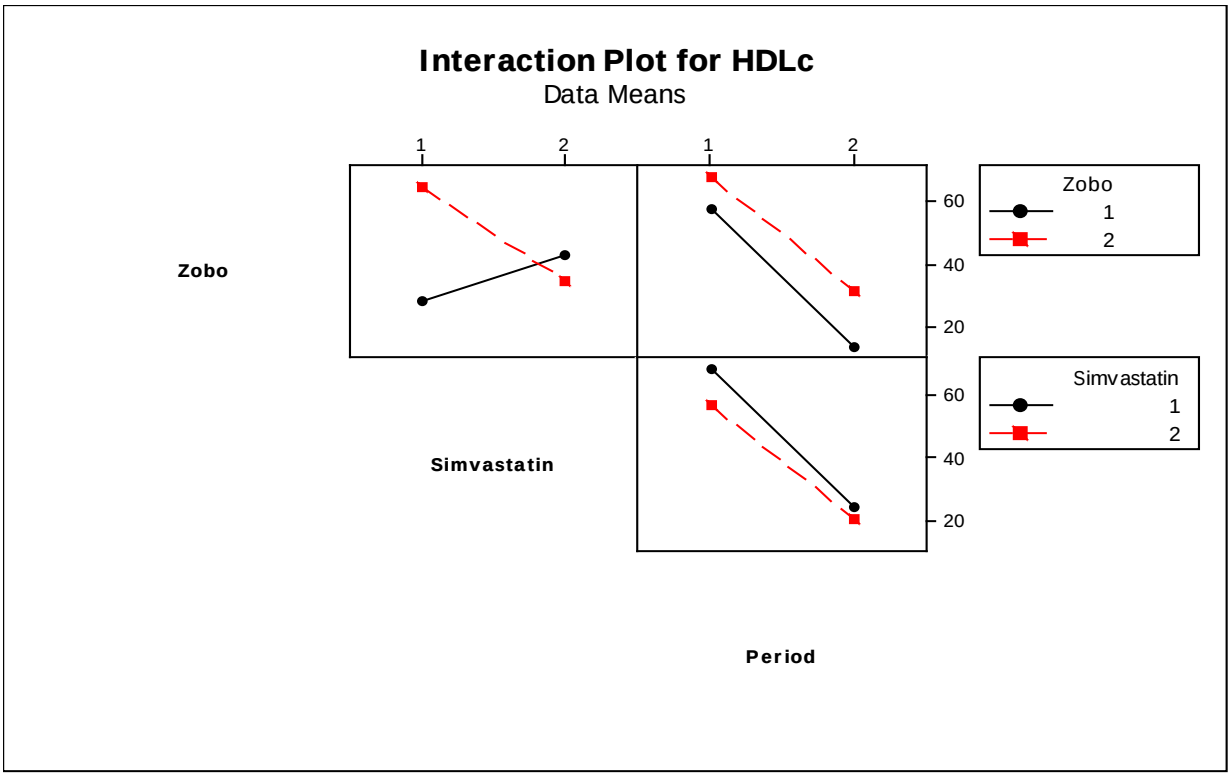


Fig 4.27: Percentage increase in high density lipoprotein cholesterol level, resulting from interactions between aqueous extract of *Hibiscus sabdariffa* (*Zobo*) and simvastatin, simvastatin and treatment period, and *Zobo* and treatment period. Number 1 and 2 represent low and high level of each factor, respectively.

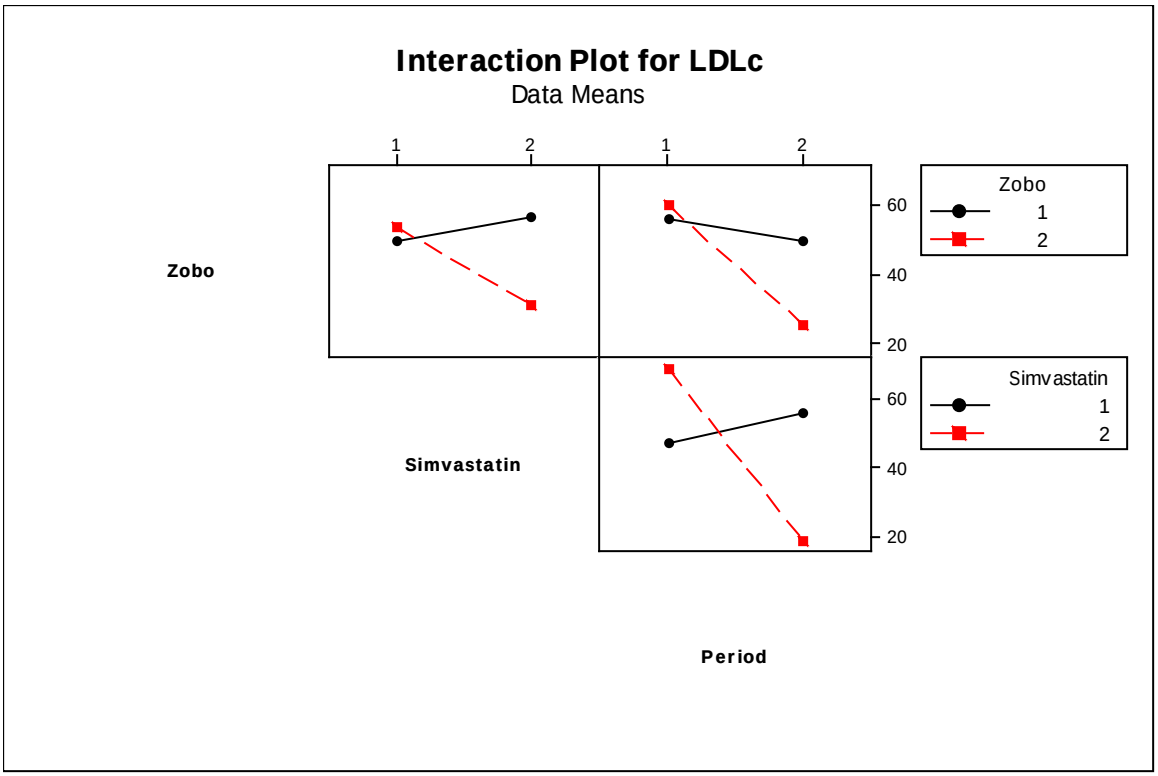


Fig 4.28: Percentage increase in low density lipoprotein cholesterol level, resulting from interactions between aqueous extract of *Hibiscus sabdariffa* (*Zobo*) and simvastatin, simvastatin and treatment period, and *Zobo* and treatment period. Number 1 and 2 represent low and high level of each factor, respectively.

CHAPTER FIVE

DISCUSSION

5.1 Discussion

The survey conducted in this study confirmed that many people believed in using aqueous beverage of *Hibiscus sabdariffa* (ABHS) for acute and chronic illnesses such as anemia, hypertension, diabetes, hyperlipidemia, liver disease, hemorrhoid, and kidney disease. Some pharmacological properties relating to folkloric use of ABHS have been confirmed by various animal and human studies. Examples include the hepatoprotective effects of extracts of *Hibiscus sabdariffa* in paracetamol-induced hepatotoxicity (Ali *et al.,* 2003) and cadmium-induced hepatotoxicity in rats (Asagba *et al.,* 2007). Animals and human studies have also substantiated the effectiveness of extracts of *Hibiscus sabdariffa* in the management of hypertension. In spontaneously hypertensive rats (Onyenekwe *et al*., 1999; Odigie *et al*., 2003) and anaesthetized rats (Adegunloye *et al.,* 1996), the extracts of *Hibiscus sabdariffa* showed antihypertensive activity in a dose-dependent manner. In human studies, the extracts of *H. sabdariffa* lowered both systolic and diastolic blood pressure by 11.0 % within 12 days (Faraji and Tarkhani, 1999), and the standardized extract and tea were as effective as captopril (Herrera-Arellano *et al.,* 2004) and lisinopril (McKay *et al.,* 2010) in patients with mild to moderate hypertension.

There are studies confirming the effectiveness of extracts of *Hibiscus sabdariffa* in lowering lipid profile parameters as reported by participants in this study. Studies in animals and human showed that the extracts of *Hibiscus sabdariffa* were effective in reducing lipid profile in fructose-fed rats (Chen *et al.,* 2004), hypercholesterolemic rats (Chen et al. 2004; Carvajal-Zarrabal *et al*., 2005; Hirunpanich *et al*., 2006), and in patients with metabolic syndrome (Gurrola-Díaz *et al.,* 2010).

Other self–reported indications by the participants include the beverage's use in improving vision, enhance sexual performance, and treat irregular menstruation, which are yet to be confirmed by either animal or human studies. Nevertheless, one of the indications for ABHS, which is relaxation, had been documented in an animal study which reported that aqueous extract of *Hibiscus sabdariffa* had anxiolytic properties (Fakeye *et al*., 2008a).

Though herbs are generally believed to be safe (Aschwanden, 2001; Holt and Chandra, 2002), studies have shown that herbs may give rise to side effects and adverse drug reactions (Barnes, 1998; Ernst, 2000). This has also been found true with the use of aqueous beverage of *Hibiscus sabdariffa*. Survey participants in this study reported diarrhea, vomiting, dizziness, insomnia, tiredness and decreased libido as some of the side effects experienced. In animal toxicity studies carried out by Fakeye (2008) and Fakeye *et al.,* (2008a), it was reported that chronic administration of high dose of aqueous extract and lower doses of the residual water-soluble fraction of the extract of *H. sabdariffa* resulted in severe weight loss accompanied by diarrhea. This partly confirmed the side effect of diarrhea experienced by some of the participants in the study. Vomiting and diarrhea, if protracted may cause volume depletion, electrolyte imbalance, disorientation, confusion, and hypovolemic shock (Boxtel *et al*., 2008; Ariens 2012; Atkinson and Huang 2012).

Evidence abounds of people co-administering medicines with juices (Huang and Lesko, 2004; Izzo, 2005) and herbs (Hansen *et al*., 2003; Fakeye *et al*., 2009; Djuv *et al*., 2013). Grape fruit juice inhibits intestinal CYP3A4 and other enzymes. When coadministered with drugs it caused 1250% and 1400% change in area under the plasma concentration-time curve (AUC) of lovastatin and simvastatin, respectively (Huang and Lesko, 2004). Apple and orange juices have also been shown to affect transporters such as P-glycoprotein and organic anion-transporting polypeptide (OATP) responsible for transporting drugs, such as cyclosporine and fexofenadine (Huang and Lesko, 2004). These interactions may affect the efficacy of the coadministered medicine, and in some cases, may cause unwanted side effects. Concomitant use of aqueous beverage of *Hibiscus sabdariffa* with conventional drugs was reported in the survey. As reported in

this study some side effects were experienced by participants as a result of coadministration of ABHS with drugs.

Previous studies on the coadministration of ABHS with some drugs showed that ABHS significantly altered some pharmacokinetic parameters of chloroquine (Mahmoud *et al*., 1994), paracetamol (Kolawole and Maduenyi, 2004) and diclofenac (Fakeye *et al.*, 2007a). As reported by many authors, herb-drug interactions are mediated by many mechanisms (Ioannides 2002; Sørensen, 2002; Izzo and Ernst, 2012). One of the possible mechanisms of herb-drug interaction is herb-induced modulation of CYP isoforms responsible for the metabolism of the concomitantly administered drug (Ioannides, 2002). Others include alteration in the rate and extent of absorption, protein binding displacement, and changes in the renal excretion of drugs (Ioannides, 2002)..

The *in vitro* study showed that extracts of *H. sabdariffa* have weak inhibitory activities on the eight selected CYP isoforms. Even though, a previous study had reported that aqueous extract of *Hibiscus sabdariffa* had no effect on the total hepatic CYP contents and on the activities of CYP isoforms like 1A1, 1A2, 2B1, 2B2, 2E1 and 3A in rats (Prommetta *et al*., 2006); this present study, having utilized both aqueous and ethanolic extracts of *Hibiscus sabdariffa* and human liver microsomes, showed that the extracts are weak inhibitors of major CYP isoforms (CYPs 1A2, 2C9, 2C19, 2D6, and 3A4). Clinically important classes of drugs such as anticonvulsants, antiretrovirals, antiinfectives, antihypertensives, and non-steroidal anti-inflammatory drugs (NSAIDs) are substrates of these CYP isoforms. Studies have shown that inhibition of CYP-mediated metabolism of a drug may lead to therapeutic failure, enhanced therapeutic effect, increased side effect, and toxicity (Rodrigues and Rushmore, 2002; Kimura *et al.*, 2010). The weak inhibitory activities of *Hibiscus sabdariffa* extracts on CYP isoforms may become clinically significant if coadministered with drugs with narrow therapeutic index or drugs that undergo capacity limited metabolism such as phenytoin, carbamazepine, theophylline, and digoxin.

The calyces of *Hibiscus sabdariffa* used in this study contain secondary metabolites such as alkaloids, cardenolides, saponins, tannins, anthocyanins and flavonoids as also reported by Gautam (2004) and Mahadevan *et al.,* (2009). These secondary metabolites

are responsible for most pharmacological activities observed with extracts of *Hibiscus sabdariffa* (Badreldin *et al*., 2005; Mahadevan *et al*., 2009; Maganha *et al*., 2010; Da-Costa-Rocha *et al.* 2014). In other studies coumarins, saponins, anthocyanins, and flavonoids have been shown to exhibit inhibitory activities on CYP isoforms (Obermeier *et al*., 1995; Kim *et al*., 1997; Henderson *et al.* 2000; Ajiboye *et al*., 2011). It is not unlikely that these secondary metabolites which are known to be present in the calyces of *Hibiscus sabdariffa* were responsible for the observed inhibitory activities of the extracts on the eight cytochrome P450 isoforms studied.

Due to numerous compounds present in the extract, molar IC_{50} values could not be determined. In order to estimate the significance of the observed inhibitions, the IC_{50} values had to be converted to L/dose unit. This is the volume to which a unit dose of the extract must be diluted to give the same *in vitro* IC_{50} values (Strandell *et al*., 2004). When representing the IC_{50} values in liters/dose units, the possibility of reaching *in vivo* concentrations that may cause significant inhibition, can be estimated. Strandell *et al.,* (2004) concluded that an *in vitro* IC_{50} value $\geq$ 5.0 L/dose may produce *in vivo* inhibition of the same enzyme. For the extracts used in this study, the *in vitro* IC_{50} values in Litre/dose were almost equal to the blood volume of an average human for CYP 2B6, 2C8, 2C19 and 2D6 while for CYP 1A2 it was slightly higher than 5.0 L. These results, according to Strandell *et al.,* (2004) suggested that the extracts are likely to cause *in vivo* inhibition of these CYP isoforms.

However, contrary to Strandel *et al.,* (2004), a single time point phenotypic metabolic ratio (PMR), in healthy volunteers showed no obvious inhibition of CYP1A2 and CYP2D6 using caffeine and metoprolol as probe substrates, respectively. This shows that, though, the *in vitro* findings suggested that the extracts of *Hibiscus sabdariffa* are weak inhibitors of the eight selected CYP isoforms, actual *in vivo* study in human showed that ABHS had no obvious inhibitory activity on CYP1A2 and 2D6.

Cytochrome P450 1A2 accounts for 15% of the total CYP isoforms in human liver and is responsible for metabolizing about 2% of drugs (Carrillo *et al.,* 2000). This enzyme is responsible for metabolizing olanzapine, clozapine, haloperidol, imipramine, clomipramine, fluvoxamine, and tacrine, either partially or solely (Bertilsson *et al*. 1994;

Spigset *et al.* 1999; Callaghan *et al.* 2012). Metoprolol on the other hand is a known metabolic probe for CYP2D6 and this CYP isoform accounts for ≈30% of total CYP isoforms present in the liver (Bertilsson *et al.,* 2002; Williams *et al*., 2004; Ingelman-Sundberg, 2005; Guengerich, 2007). Though, polymorphic in nature and shows inter-individual variability, CYP2D6 is responsible for the metabolism of clinically important drugs like tricyclic antidepressants, carvedilol, chlorpromazine, codeine, diphenhydramine, tamoxifen, and timolol (Bertilsson *et al.* 2002; Ingelman-Sundberg, 2005). A complete lack of this enzyme may cause toxic reactions or adverse drug reaction while excessive expression may cause treatment failure. The apparent lack of inhibitory activity of the ABHS on CYP1A2 and CYP2D6 suggests that the coadministration of the beverage with drugs that are substrates of these enzymes may not pose any significant risk. The disparity in the *in vitro* and *in vivo* inhibitory activities of *Hibiscus sabdariffa* may be due to low bioavailability of secondary metabolites in humans as reported by Bhattaram *et al.,* (2002) or the fact that secondary metabolites have short elimination half-lives *in vivo* (Wu *et al.,* 2002; Yu *et al.,* 2012).

Despite the lack of metabolic inhibition of CYP1A2 and CYP2D6 using PMR, aqueous beverage of *Hibiscus sabdariffa* affected pharmacokinetic parameters of caffeine and simvastatin such as the absorption rate constant (K_a), absorption rate half-life ($t_{1/2}K_a$), elimination rate constant (K_e), time to reach maximum plasma concentration (T_{max}), maximum plasma concentration (C_{max}) and apparent clearance (CL/F) in human. The beverage caused approximately one-third decrease in absorption rate constant and one-fifth increase in elimination rate constant without a corresponding change in the apparent clearance of caffeine. These changes did not affect the extent of absorption of the drug. The T_{max} and C_{max} of caffeine were also increased slightly. Though, these observed changes were not statistically significant, Food and Drug Agency (2012) and European Medicine Agency (2014) stated that any change of 25% and above, for parameters such as C_{max}, $AUC_{0-\infty,}$ K_e, and CL, could be clinically relevant even though it may not be statistically significant. In this study, the observed magnitude of change in some of the pharmacokinetic parameters of caffeine when taken with ABHS may be clinically relevant. Also, ABHS decreased the elimination rate constant of simvastatin and decreased its clearance by more than half. The time to reach peak plasma concentration

and the mean resident time of the drug were also increased. The changes in the pharmacokinetic parameters of simvastatin may lead to its accumulation in the body if coadministered with ABHS. This may cause increased side effect and may also improve the therapeutic effect of the drug.

As mentioned earlier, previous clinical studies reported a decrease in the terminal half-life of acetaminophen without a significant corresponding change in AUC and clearance (Kolawole and Maduenyi, 2004). This is similar with the findings in this study where the terminal half-life of caffeine was decreased without a corresponding decrease in apparent clearance. A reduction of more than 50% in the apparent clearance of simvastatin was also observed which is similar to the reduction in the metabolic clearance of diclofenac as reported by Fakeye *et al.,* (2007a). These drugs are metabolised by more than one CYP isoform. Paracetamol is metabolised by CYP isoforms 1A2, 2A6, 2D6, 2E1 and 3A4 (Raucy *et al.,* 1989; Dong *et al.,* 2000); diclofenac by CYP isoforms 2C9 and 3A4 (Shen *et al.,* 1999; Tang *et al.,* 1999) and caffeine by 1A2 and 2E1 while simvastatin is metabolised majorly by 3A4/5 (Lilja *et al*., 2000; Prueksaritanont *et al*., 2003) Drugs metabolised by multiple CYP isoforms or drugs with other dominant metabolic pathways such as glucoronidation and sulphonation may not be significantly affected by inhibitors of single CYP isoform except if the inhibitor inhibits the multiple CYP isoforms involved in the metabolic pathways of the drug concerned. However, a potent inhibitor of most or all the CYP isoforms responsible for the metabolism of a drug may significantly affect the drug's metabolic clearance. All the isoforms involved in the metabolism of simvastatin, caffeine, acetaminophen and diclofenac are weakly inhibited by the extracts of *H. sabdariffa in vitro*. While this may seem to explain the reduction in the metabolic clearance of simvastatin and diclofenac as a result of possibly mild inhibition, it cannot be proffered as the reason for the reduction in the elimination of paracetamol and the increase in the elimination of caffeine. The findings in the *in vivo* study proved that these effects of ABHS on the pharmacokinetic parameters of caffeine and simvastatin were apparently not mediated by inhibition of the cytochrome P450 enzymes. It is not impossible that the observed herb-drug interactions were caused by other mechanisms apart from metabolic inhibition. The beverage may be causing herb-drug interactions by alteration in the gastrointestinal motility and functions which could lead to decreased or

increased absorption of drugs administered concomitantly. Aqueous beverage of *Hibiscus sabdariffa* used with caffeine and simvastatin had a pH of 2.5. Since caffeine and simvastatin are weak bases and administered by volunteers in a fasted state, it is also likely that the drugs were mostly ionized in the acidic gastric medium, reducing the absorption rate of these drugs as observed in this study. This possible mechanism could also explain why the time to reach peak plasma concentration in both drugs was extended with ABHS. It has been reported that *Hibiscus sabdariffa* inhibits gastrointestinal motility delaying the absorption of drugs in the small intestine (Owulade *et al*., 2004; Sarkar *et al.* 2012; Eiman *et al*., 2014). This further buttresses the increase in time to reach maximum concentration for both simvastatin and caffeine. Other mechanisms could be the modulation of transport proteins like P-glycoprotein and herb induced changes in the renal elimination of coadministered drugs.

There have been reported cases of people using herbs alongside their medications (Leape *et al.,* 1991; Begbie *et al.,* 1996; Barnes *et al.,* 2004; Fakeye *et al.,* 2009; Djuv *et al.,* 2013) which may lead to synergism, potentiation, additive or antagonistic effect. From the survey, aqueous beverage of *H. sabdariffa* is used in administering medications like antihypertensives, oral hypoglycemic agents, and antilipidemic drugs. Previous studies have suggested that extracts of *Hibiscus sabdariffa* possess many pharmacological activities such as antihyptensive, hypoglycemic and antilipidemic activities. From the present study, combinations of low-dose-AEHS + low-dose-simvastatin, and high-dose-AEHS + high-dose-simvastatin improved the values of total cholesterol (T_c,) triglyceride (TG) and high density lipoprotein cholesterol (HDL_c) within a period of two weeks when compared to low-dose-simvastatin given alone. These findings, in an animal study which suggests a possible herb-drug interaction may partly be explained by the effect of ABHS on the pharmacokinetic parameters of simvastatin in healthy human volunteers as reported in this study. Aqueous beverage of *H. sabdariffa* was observed to moderately affect elimination rate constant and the mean resident time of simvastatin while highly reducing its apparent clearance, partly explaining the better antilipidemic effect observed with addition of ABHS to simvastatin when compared to simvastatin alone.

Simvastatin, a pro-drug, is extensively metabolised by the intestinal and hepatic CYP3A4 to its active metabolite, simvastatin hydroxyacid (Sugimoto, 2001; Prueksaritanont *et al.*, 2003) which is responsible for the inhibition of HMG-CoA, an enzyme involved in the rate limiting step in cholesterol synthesis. An increase in the level of simvastatin will lead to an increase in the production of its active metabolite which in turn will cause further improvement in the lipid profile as observed when low-dose-AEHS was combined with low-dose-simvastatin. This is feasible because from the pharmacokinetic study reported, ABHS reduced the elimination of simvastatin with a corresponding decrease in its apparent clearance. It also significantly increased the mean resident time of simvastatin in the body thus leading to the accumulation of simvastatin and a consequent increase in the production of its metabolite. This was most likely responsible for the reduction in the level of total cholesterol which probably led to increased synthesis of low density lipoprotein cholesterol (LDL_c) receptors and increased clearance of LDL_c from systemic circulation (Brown and Goldstein, 1986; Costet, 2010). Increased level of simvastatin will also lead to decrease in the synthesis of triglycerides (Schachter, 2005). Other reasons for the significant improvement in lipid profile when ABHS was coadministered may be due to the multiple mechanisms of action of AEHS on lipid profile coupled with that of simvastatin that effected an immediate lowering of these parameters. These combinations, especially the combination of low doses of AEHS and simvastatin, could be used in cases where high dose of simvastatin is contraindicated since statins-induce myopathy is dose dependent (Niemi, 2010), or when immediate lowering of these lipid profiles are necessary.

Individual administration of the AEHS and simvastatin were effective in lowering total cholesterol (T_c), triglyceride (TG) and low density lipoprotein cholesterol (LDL_c) and also increasing high density lipoprotein cholesterol (HDL_c). These results are similar to other *in vivo* studies in hyperlipidemic animal models, in healthy human volunteers and individuals with metabolic syndrome (Lin *et al.*, 2007; Agoreyo *et al.*, 2008; Gurrola-Díaz *et al.*, 2010b). However, the Nature (N) of the substance (whether drug or herb) used in the treatment was more important for T_c than other factors. Comparatively, the herb, AEHS, gave a better reduction in T_c than simvastatin even when the doses were increased as noticed in the interaction between Nature and Dose. Simvastatin has been

shown to reduce T_c as much as 30%, which is similar to the findings in this study, by decreasing the production of cholesterol and increasing the capacity of the liver to remove LDL_c from circulation (Costet, 2010). But in addition to these mechanisms, *H. sabdariffa* also inhibits lipid peroxidation through its anthocyanin and proteocathechuic contents (Hirunpanich *et al.*, 2006). It has also been reported that the polyphenols in *H. sabdariffa* reduce total cholesterol, in a dose-dependent manner through mechanisms that decrease the expression of fatty acid synthase and HMG CoA reductase (Hopkins *et al.*, 2013). These mechanisms might explain why there was a better reduction in T_c by AEHS than simvastatin.

Monitoring reduction in T_c, LDL_c and increase in HDL_c is essential to slowing down the progression of Coronary Heart Disease (CHD). Risk of CHD is strongly correlated with LDL_c and HDL_c rather than T_c (Sharrett *et al.*, 2001) but elevated T_c singly predicts CHD and death [National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) 2002]. Also, a 38.67 mg/dL decrease in T_c is associated with 25% reduction in CVD risk (Austin *et al.*, 1998, 2000). Since AEHS is more effective than simvastatin in lowering T_c in the animal model, it may be preferred in reducing incidence of CHD, though further studies need to be done in humans to establish this.

In clinical trials and other studies simvastatin has been shown to consistently decrease TG, LDL_c and T_c while increasing HDL_c (Wright and Flapan, 1994; Thomas *et al.*, 2005) but in this study, however the interaction between nature and treatment period showed that simvastatin caused a further decrease in TG at 2 weeks, but reversal of effect on HDL_c and LDL_c. with longer period of treatment (4 weeks). For AEHS, animal studies lasting from 4 – 9 weeks showed that the treatment period does not significantly change the effect of *H. sabdariffa* extract on lipid profiles (El-Saadany *et al.*, 1991; Lin *et al.*, 2007; Kuriyan *et al.*, 2010; Gurrola-Díaz *et al.*, 2010). These agree with the findings in this study.

5.2 Limitations

The limitations in this study include:

The participants in the survey on the pattern and extent of use of aqueous beverage of *Hibiscus sabdariffa* might not be representative of the Nigerian population. Also, the cross-sectional study design of this survey may affect the extrapolation and the generalizability of the findings. Most of the participants were literates and members of the university community who may be illiterate were excluded.

The extrapolation of *in vitro* findings to *in vivo* is limited by the use of clinically relevant substrate and inhibitors. For the *in vitro* study, Food and Drug Administration (2012) recommended substrates and inhibitors were used while for the *in vivo* study, recommended substrates were used for CYP1A2 and CYP2D6 but simvastatin was used instead of midazolam for CYP3A4. Simvastatin had been used in some studies as substrates of CYP3A4. Other limitations of *in vitro* to *in vivo* extrapolation include supratherapeutic herb concentration which may produce inhibition *in vitro* but not *in vivo*, underestimation of IC_{50} due to protein binding at high microsomal protein content, choice of *in vitro* enzyme system and the genotype or phenotype metabolic activity of the liver donor(s).

The use of two *in vivo* CYP isoform substrates obviously limits the generalization of the findings to all CYP metabolism enzymes.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Aqueous beverage of *Hibiscus sabdariffa* is extensively used by people of diverse educational, religious, and sociocultural background for various purposes. these include its co-use with a wide range of drugs, some of which are medications for chronic diseases. This practice may inadvertently expose the user to untoward effects and herb-drug interactions. A compilation of the side effects and deliberate education of the populace on possible herb-drug interactions with concomitant use of the beverage with conventional drugs may go a long way to minimize side effects and prevent unwanted herb-drug interaction.

Aqueous beverage of *Hibiscus sabdariffa* is not an obvious inhibitor of CYP1A2 and CYP2D6 in humans but its extracts are weak *in vitro* inhibitors of eight cytochrome P450 isoforms responsible for the metabolism of more than 80% of drugs. Though, it poses no risk of producing CYP1A2 and CYP2D6 metabolism-mediated herb-drug interactions in humans, it however produced important herb-drug interactions by affecting the processes of absorption and elimination of caffeine and simvastatin, warranting a deliberate policy and warning on its co-use with drug with similar properties with caffeine and simvastatin.

Aqueous beverage of *Hibiscus sabdariffa* greatly reduced the apparent clearance of simvastatin and increased its mean resident time while concurrent administration of low doses of aqueous extract of *Hibiscus sabdariffa* and simvastatin resulted in better reduction in lipid profile at short duration of treatment than low dose simvastatin used alone. These herb-drug interactions may be beneficial in the treatment of hyperlipidemia where aqueous beverage of *Hibiscus sabdariffa* could be used as adjunct therapy for

immediate improvement in lipid profile or in patients who are at great risk of developing coronary heart disease.

6.2 Recommendations and SuggestionS for further work

There is need for educational intervention on the use of aqueous beverage of *Hibiscus sabdariffa* to administer medication as this practice may lead to both beneficial and non-beneficial herb-drug interactions and adverse drug reactions. Also, product inserts detailing the self-reported side effects experienced with the use of aqueous beverage of *Hibiscus sabdariffa* should be used in commercialized products of the beverage.

The use of aqueous beverage of *Hibiscus sabdariffa* or its combination with simvastatin at low doses may be considered in cases of hyperlipidemia requiring immediate improvement and in patients prone to the development of myopathy at high doses of simvastatin.

Aqueous beverage of *Hibiscus sabdariffa* may be coadministered with drugs that are substrates of CYP1A2 and CYP2D6 isoforms; however, caution should be exercised since the beverage could affect the pharmacokinetic parameters of the coadministered drugs. Caution should also be exercised when co-administering aqueous beverage of *Hibiscus sabdariffa* with caffeine or simvastatin since it is now known that the beverage affects their pharmacokinetic parameters appreciably.

There is need for extensive study on other mechanisms that may be responsible for the herb-drug interactions with aqueous beverage of *Hibiscus sabdariffa* as reported in this study. Possible mechanisms that may be explored include its effect on P-glycoprotein, intestinal absorption of drugs and alteration in the renal excretion of drugs.

There is also need for standardization of the preparation of water beverage of *Hibiscus sabdariffa* to ensure uniformity of dose and findings.

6.3 Contributions to knowledge

To the best of my knowledge, this study shows that aqueous and ethanolic extracts of *Hibiscus sabdariffa* have some inhibitory activity on eight selected cytochrome P450 enzymes responsible for the metabolism of over 70% of drugs.

Though, aqueous beverage of *Hibiscus sabdariffa* did not show *in vivo* inhibitory activities on CYP1A2 and CYP2D6 as indicated by phenotypic metabolic ratio of caffeine and metoprolol. The absorption and elimination of caffeine and simvastatin was however affected this therefore underscore the need for caution in coadministering aqueous beverage of *Hibiscus sabdariffa* with caffeine and simvastatin as well as other related drugs.

In addition, aqueous extract of *Hibiscus sabdariffa* lowers total cholesterol better than simvastatin and concomitant administration of low-dose aqueous extract of *Hibiscus sabdariffa* with low-dose simvastatin was more effective in lowering total cholesterol, triglyceride, and high density lipoprotein cholesterol. Thus combination of low doses of *Hibiscus sabdariffa* and simvastatin could be employed in the treatment of hyperlipidemia and atherosclerosis requiring high dose of simvastatin.

REFERENCES

Adegunloye, B. J., Omoniyi, J. O., Owolabi, O. A., Ajagbonna, O. P., Sofola, O. A., and Coker, H. A. 1996. Mechanisms of the blood pressure lowering effect of the calyx extract of *Hibiscus sabdariffa* in rats. *African Journal of Medicine and Medical Sciences* 25.3: 235–238.

Adidala, R. R., Arrabelli, M., Regula, J., and Nakka, S. 2014. Development and validation of RP-HPLC method for simvastatin using lovastatin as internal standard. *Journal of Drug Delivery and Therapeutics* 4.3: 171–174.

Agbor, G. A., Moumbegna, P., Oluwasola, E. O., Nwosu, L. U., Njoku, R.-Cc, Kanu, S., Emekabasi, E. I., Akin, F., Obasi, A. P. and Abudei, F. A. 2011. Antioxidant capacity of some plants foods and beverages consumed in the eastern region of Nigeria. *African Journal of Traditional, Complementary and Alternative Medicines* 8.4:362-369 Retrieved Sept. 18, 2016, from http://www.ajol.info/index.php/ajtcam/article/view/66950.

Agoreyo, F. O., Agoreyo, B. O., and Onuorah, M. N. 2008. Effect of aqueous extracts of *Hibiscus sabdariffa* and *Zingiber Officinale* on blood cholesterol and glucose levels of rats. *African Journal of Biotechnology* 7.21: 3949-3951.

Ajiboye, T. O., Salawu, N. A., Yakubu, M. T., Oladiji, A. T., Akanji, M. A., and Okogun, J. I. 2011. Antioxidant and drug detoxification potentials of *Hibiscus sabdariffa* anthocyanin extract. *Drug and chemical toxicology* 34.2: 109–115.

Akindahunsi, A. A., and Olaleye, M. T. 2003. Toxicological investigation of aqueous-methanolic extract of the calyces of *Hibiscus sabdariffa* L. *Journal of Ethnopharmacology* 89.1: 161–164.

Ali, B. H., Mousa, H. M., and El-Mougy, S. 2003. The effect of a water extract and anthocyanins of *Hibiscus sabdariffa* L. on paracetamol-induced hepatoxicity in rats. *Phytotherapy Research* 17.1: 56–59.

Ali, B. H., Wabel, N. A., and Blunden, G. 2005. Phytochemical, pharmacological, and toxicological aspects of *Hibiscus sabdariffa* L.: a review. *Phytotherapy Research* 19.5: 369–375.

American Psychological Association. 1986. Guidelines for ethical conduct in the care and use of animals. *Journal of the Experimental Analysis of Behavior* 45.2: 127–132.

Aninat, C., Piton, A., Glaise, D., Le Charpentier, T., Langouët, S., Morel, F., Guguen-Guillouzo C., and Guillouzo, A. 2006. Expression of cytochromes P450, conjugating enzymes and nuclear receptors in human hepatoma HepaRG cells. *Drug Metabolism and Disposition* 34.1: 75–83.

Aoyama, T., Yamano, S., Guzelian, P. S., Gelboin, H. V., and Gonzalez, F. J. 1990. Five of 12 forms of vaccinia virus-expressed human hepatic cytochrome P450 metabolically activate aflatoxin B1. *Proceedings of the National Academy of Sciences* 87.12: 4790–4793.

Ariens, E. J. 2012. Molecular Pharmacology V3: The Model of Action of Biology Active Compounds.1st edition, USA. Elsevier. 38-48 Retrieved Nov. 27, 2016, from https://books.google.com.pe/books?id=eHDg0WV98XcC&q=active+transport#v=snippet&q=active%20transport&f=false

Armstrong, D. 2004. Gastric pH — the most relevant predictor of benefit in reflux disease. *Alimentary Pharmacology and Therapeutics* 20.s5:19–26.

Asagba, S. O., Adaikpoh, M. A., Kadiri, H., and Obi, F. O. 2007. Influence of aqueous extract of *Hibiscus sabdariffa* L. petal on cadmium toxicity in rats. *Biological Trace Element Research* 115.1: 47–57.

Aschwanden, C. 2001. Herbs for health, but how safe are they? *Bulletin of the World Health Organization* 79.7: 691–692.

Atkinson, A. J., and Huang, S. M. 2012. Principles of Clinical Pharmacology. USA. Elservier. 42-44 Retrieved Nov. 27, 2016, from https://books.google.com.ng/books?id=ei_TnqM5z24C&pg=PA236&lpg=PA236

&dq=Atkinson,+A.+J.,+and+Huang,+S.+M.+2012.+Principles+of+Clinical+Pharmacology.&source=bl&ots=2PZDqGMWqT&sig=XjotezBk7ivC_g0rc_3dFPFIr40&hl=en&sa=X&ved=0ahUKEwj2_PSb1cjQAhXoLMAKHQ4dDIEQ6AEIMjAF#v=onepage&q=gastric%20motility&f=false

Aurelio, D. L., Edgardo, R. G., and Navarro-Galindo, S. 2008. Thermal kinetic degradation of anthocyanins in a roselle (*Hibiscus sabdariffa* L. cv.“Criollo”) infusion. *International Journal of Food Science and Technology* 43.2: 322–325.

Austin, M. A., Hokanson, J. E., and Edwards, K. L. 1998. Hypertriglyceridemia as a cardiovascular risk factor. *The American Journal of Cardiology* 81.4: 7B–12B.

Austin, M. A., McKnight, B., Edwards, K. L., Bradley, C. M., McNeely, M. J., Psaty, B. M., Brunzell, J. D., and Motulsky, A. G. 2000. Cardiovascular Disease Mortality in Familial Forms of Hypertriglyceridemia: A 20-Year Prospective Study. *Circulation* 101.24: 2777–2782.

Back, D. J., Houlgrave, R., Tjia, J. F., Ward, S., and Orme, M. L. 1991. Effect of the progestogens, gestodene, 3-keto desogestrel, levonorgestrel, norethisterone and norgestimate on the oxidation of ethinyloestradiol and other substrates by human liver microsomes. *The Journal of Steroid Biochemistry and Molecular Biology* 38.2: 219–225.

Backman, J. T., Kyrklund, C., Neuvonen, M., and Neuvonen, P. J. 2002. Gemfibrozil greatly increases plasma concentrations of cerivastatin. *Clinical Pharmacology and Therapeutics* 72.6: 685–691.

Bajpai, M., Roskos, L. K., Shen, D. D., and Levy, R. H. 1996. Roles of cytochrome P4502C9 and cytochrome P4502C19 in the stereoselective metabolism of phenytoin to its major metabolite. *Drug Metabolism and Disposition: The Biological Fate of Chemicals* 24.12: 1401–1403.

Barnes, J. 1998. Adverse effects of herbal drugs. *Focus on Alternative and Complementary Therapies* 33: 128–128.

Barnes, P. M., Powell-Griner, E., McFann, K., and Nahin, R. L. 2004. Complementary and alternative medicine use among adults: United States, 2002. *Seminars in Integrative Medicine*, Elsevier, 54–71. Retrieved Feb. 13, 2016, from http://www.sciencedirect.com/science/article/pii/S1543115004000389,

Begbie, S. D., Kerestes, Z. L., and Bell, D. R. 1996. Patterns of alternative medicine use by cancer patients. *The Medical Journal of Australia* 16510: 545–548.

Bertilsson, L., Carrillo, J. A., Dahl, M. L., Llerena, A., Alm, C., Bondesson, U., Lindström, L., Rodriguez de la Rubia, I., Ramos, S., and Benitez J. 1994. Clozapine disposition covaries with CYP1A2 activity determined by a caffeine test. *British Journal of Clinical Pharmacology* 385: 471–473.

Bertilsson, L., Dahl, M.-L., Dalén, P., and Al-Shurbaji, A. 2002. Molecular genetics of CYP2D6: clinical relevance with focus on psychotropic drugs. *British Journal of Clinical Pharmacology* 53.2: 111–122.

Bertz, R. J., and Granneman, G. R. 1997. Use of *in vitro* and *in vivo* data to estimate the likelihood of metabolic pharmacokinetic interactions. *Clinical Pharmacokinetics* 32.3: 210–258.

Bhattaram, V. A., Graefe, U., Kohlert, C., Veit, M., and Derendorf, H. 2002. Pharmacokinetics and bioavailability of herbal medicinal products. *Phytomedicine* 9: 1–33.

Bjornsson, T. D., Callaghan, J. T., Einolf, H. J., Fischer, V., Gan, L., Grimm, S., Kao, J., King, S, P., Miwa, G., Ni, L., Kumar, G., McLeod, J., Obach, R, S., Roberts, S., Roe, A., Shah, A., Snikeris, F., Sullivan, J, T., Tweedie, D., Vega, J, M., Walsh, J., Wrighton, S, A.; Pharmaceutical Research and Manufacturers of America (PhRMA) Drug Metabolism/Clinical Pharmacology Technical Working Group; and FDA Center for Drug Evaluation and Research (CDER). 2003. The conduct of *in vitro* and *in vivo* drug-drug interaction studies: a Pharmaceutical Research and Manufacturers of America (PhRMA) perspective. *Drug Metabolism and Disposition* 31.7: 815–832.

Bonate, P. L., Reith, K., and Weir, S. 1998. Drug interactions at the renal level. Implications for drug development. *Clinical Pharmacokinetics* 34.5: 375–404.

Boxtel, C. J. van, Santoso, B., and Edwards, I. R. 2008. Drug Benefits and Risks: International Textbook of Clinical Pharmacology. The Uppsala monitoring centre - Revised 2nd Edition.. IOS Press. Amsterdam page 247-261. Retrieved Nov. 27, 2016, from https://books.google.com.ng/books?id=xQqVbRqA2N8C&printsec=frontcover&dq=Drug+Benefits+and+Risks:+International+Textbook+of+Clinical+Pharmacology&hl=en&sa=X&ved=0ahUKEwi989LC2cjQAhUKCBoKHUAYDvIQ6AEIGzAA#v=onepage&q=pharmacodynamic%20drug%20interactions&f=false

Bozikas, V. P., Papakosta, M., Niopas, I., Karavatos, A., and Mirtsou-Fidani, V. 2004. Smoking impact on CYP1A2 activity in a group of patients with schizophrenia. *European Neuropsychopharmacology* 14.1: 39–44.

Bressler, R. 2005. Herb-drug interactions: interactions between *Ginkgo biloba* and prescription medications. *Geriatrics* 60.4: 30–33.

Brown, M. S., and Goldstein, J. L. 1986. A receptor-mediated pathway for cholesterol homeostasis. *Science* 232.4746: 34–47.

Burget, D. W., Chiverton, S. G., and Hunt, R. H. 1990. Is there an optimal degree of acid suppression for healing of duodenal ulcers? A model of the relationship between ulcer healing and acid suppression. *Gastroenterology* 99.2: 345–351.

Burnham, B. E. 1995. Garlic as a possible risk for postoperative bleeding. *Plastic and Reconstructive Surgery* 95.1: 213.

Button, W. G., Judson, P. N., Long, A., and Vessey, J. D. 2003. Using absolute and relative reasoning in the prediction of the potential metabolism of xenobiotics. *Journal of Chemical Information and Computer Sciences* 43.5: 1371–1377.

Callaghan, D. J. T., Bergstrom, R. F., Ptak, L. R., and Beasley, C. M. 2012. Olanzapine. *Clinical Pharmacokinetics* 373: 177–193.

Caro, A. A., and Cederbaum, A. I. 2004. Oxidative stress, toxicology, and pharmacology of CYP2E1*. *Annual Review of Pharmacology and Toxicology* 44: 27–42.

Carrillo, J. A., Christensen, M., Ramos, S. I., Alm, C., Dahl, M. L., Benítez, J., and Bertilsson, L. 2000. Evaluation of caffeine as an *in vivo* probe for CYP1A2 using measurements in plasma, saliva, and urine. *Therapeutic Drug Monitoring* 22.4: 409-417.

Carvajal-Zarrabal, O., Waliszewski, S. M., Barradas-Dermitz, D. M., Orta-Flores, Z., Hayward-Jones, P. M., Nolasco-Hipólito, C., Angulo-Guerrero O., Sánchez-Ricaño R., Infanzón R, M., and Trujillo P, R. 2005. The consumption of *Hibiscus sabdariffa* dried calyx ethanolic extract reduced lipid profile in rats. *Plant Foods for Human Nutrition* 60.4: 153–159.

Chan, E. W. C., Lim, Y. Y., Chong, K. L., Tan, J. B. L. and Wong, S. K. 2010. Antioxidant properties of tropical and temperate herbal teas. *Journal of Food Composition and Analysis* 23.2: 185–189.

Chang, Y. C., Huang, H. P., Hsu, J. D., Yang, S. F., and Wang, C. J. 2005. Hibiscus anthocyanins rich extract-induced apoptotic cell death in human promyelocytic leukemia cells. *Toxicology and Applied Pharmacology* 205.3: 201–212.

Chang, Y. C., Huang, K. X., Huang, A. C., Ho, Y. C., and Wang, C. J. 2006. Hibiscus anthocyanins-rich extract inhibited LDL oxidation and oxLDL-mediated macrophages apoptosis. *Food and Chemical Toxicology* 44.7: 1015–1023.

Chen, C, C., Chou, F, P., Ho, Y, C., Lin, W, L., Wang, C, P., Kao, E, S., Huang, A, C., and Wang, C, J. 2004. Inhibitory effects of *Hibiscus sabdariffa* L extract on low-density lipoprotein oxidation and anti-hyperlipidemia in fructose-fed and cholesterol-fed rats. *Journal of the Science of Food and Agriculture* 8415: 1989–1996.

Chen, Y, J., Jin, Y. M., Sophie, M. V., and Kenny, J. R. 2012. Application of IVIVE and PBPK modeling in prospective prediction of clinical pharmacokinetics: strategy

and approach during the drug discovery phase with four case studies. *Biopharmaceutics and Drug Disposition* 33.2: 85–98.

Christensen, M., Andersson, K., Dalén, P., Mirghani, R. A., Muirhead, G. J., Nordmark, A., Tybring, G., Wahlberg, A., Yaşar, U., and Bertilsson, L. 2003. The karolinska cocktail for phenotyping of five human cytochrome P450 enzymes. *Clinical Pharmacology and Therapeutics* 73.6: 517–528.

Chu, X., Bleasby, K., and Evers, R. 2013. Species differences in drug transporters and implications for translating preclinical findings to humans. *Expert Opinion on Drug Metabolism and Toxicology* 9.3: 237–252.

Clement, Y. N., Williams, A. F., Aranda, D., Chase, R., Watson, N., Mohammed, R., Stubbs, O., Williamson, D. 2005. Medicinal herb use among asthmatic patients attending a specialty care facility in Trinidad. *BMC Complementary and Alternative Medicine* 5: 3. 1-8

Coecke, S., Ahr, H., Blaauboer, B. J., Bremer, S., Casati, S., Castell, J., Combes, R., Corvi, R., Crespi, C, L., Cunningham, M, L., Elaut, G., Eletti, B., Freidig, A., Gennari, A., Ghersi-Egea, J, F., Guillouzo, A., Hartung, T., Hoet, P., Ingelman-Sundberg, M., Munn, S., Janssens, W., Ladstetter, B., Leahy, D., Long, A., Meneguz, A., Monshouwer, M., Morath, S., Nagelkerke, F., Pelkonen, O., Ponti, J., Prieto, P., Richert, L., Sabbioni, E., Schaack, B., Steiling, W., Testai, E., Vericat, J, A., and Worth, A. 2006. Metabolism: a bottleneck in *in vitro* toxicological test development. The report and recommendations of ECVAM workshop 54. *Alternatives to Laboratory Animals* 34.1: 49.

Costet, P. 2010. Molecular pathways and agents for lowering LDL-cholesterol in addition to statins. *Pharmacology and Therapeutics* 1263: 263–278.

Court, M, H., Duan, S. X., Hesse, L. M., Venkatakrishnan, K., and Greenblatt, D. J. 2001. Cytochrome P-450 2B6 is responsible for interindividual variability of propofol hydroxylation by human liver microsomes. *The Journal of the American Society of Anesthesiologists* 94.1: 110–119.

Da-Costa-Rocha, I., Bonnlaender, B., Sievers, H., Pischel, I., and Heinrich, M. 2014. *Hibiscus sabdariffa* L. – A phytochemical and pharmacological review. *Food Chemistry* 165: 424–443.

Dafallah, A. A., and Al-Mustafa, Z. 1996. Investigation of the anti-inflammatory activity of *Acacia nilotica* and *Hibiscus sabdariffa*. *The American Journal Of Chinese Medicine* 24.3-4: 263–269.

Daly, A. K. 2004. Pharmacogenetics of the cytochromes P450. *Current Topics in Medicinal Chemistry* 4.16: 1733–1744.

Damle, B. D., Mummaneni, V., Kaul, S., and Knupp, C. 2002. Lack of effect of simultaneously administered didanosine encapsulated enteric bead formulation (Videx EC) on oral absorption of indinavir, ketoconazole, or ciprofloxacin. *Antimicrobial Agents and Chemotherapy* 462: 385–391.

de Graaf, C., Vermeulen, N. P., and Feenstra, K. A. 2005. Cytochrome P450 in silico: an integrative modeling approach. *Journal of Medicinal Chemistry* 48.8: 2725–2755.

de Groot, M. J., and Ekins, S. 2002. Pharmacophore modeling of cytochromes P450. *Advanced Drug Delivery Reviews* 54.3: 367–383.

de Leon, J. 2004. Psychopharmacology: atypical antipsychotic dosing: the effect of smoking and caffeine. *Psychiatric Services* 55.5:491-493.

Deli, M. A. 2009. Potential use of tight junction modulators to reversibly open membranous barriers and improve drug delivery. *Biochimica et Biophysica Acta (BBA) - Biomembranes* 1788.4: 892–910.

Ding, X., and Kaminsky, L. S. 2003. Human extrahepatic cytochromes P450: Function in xenobiotic metabolism and tissue-selective chemical toxicity in the respiratory and gastrointestinal tracts. *Annual Review of Pharmacology and Toxicology* 43.1: 149–173.

Djuv, A., Nilsen, O. G., and Steinsbekk, A. 2013. The co-use of conventional drugs and herbs among patients in Norwegian general practice: a cross-sectional study. *BMC Complementary and Alternative Medicine* 13.1: 295.

Dong, H., Haining, R. L., Thummel, K. E., Rettie, A. E., and Nelson, S. D. 2000. Involvement of human cytochrome P450 2D6 in the bioactivation of acetaminophen. *Drug Metabolism and Disposition* 28.12: 1397–1400.

Døssing, M., Pilsgaard, H., Rasmussen, B. and Poulsen, H. E. 1983. Time course of phenobarbital and cimetidine mediated changes in hepatic drug metabolism. *European Journal of Clinical Pharmacology* 25.2: 215–222.

Draper, A. J., Madan, A., and Parkinson, A. 1997. Inhibition of coumarin 7-hydroxylase activity in human liver microsomes. *Archives of Biochemistry and Biophysics* 34.11: 47–61.

Dreiseitel, A., Schreier, P., Oehme, A., Locher, S., Hajak, G., and Sand, P. G. 2008. Anthocyanins and their metabolites are weak inhibitors of cytochrome P450 3A4. *Molecular Nutrition and Food Research* 52.12: 1428–1433.

Dresser, G. K., Bailey, D. G., Leake, B. F., Schwarz, U. I., Dawson, P. A., Freeman, D. J., and Kim RB. 2002. Fruit juices inhibit organic anion transporting polypeptide–mediated drug uptake to decrease the oral availability of fexofenadine. *Clinical Pharmacology and Therapeutics* 71.1: 11–20.

Ducharme, J., and Farinotti, R. 1996. Clinical pharmacokinetics and metabolism of chloroquine. *Clinical Pharmacokinetics* 31.4: 257–274.

Edwards, R. J., Price, R. J., Watts, P. S., Renwick, A. B., Tredger, J. M., Boobis, A. R., and Lake, B, G. 2003. Induction of cytochrome P450 enzymes in cultured precision-cut human liver slices. *Drug Metabolism and Disposition* 31.3: 282–288.

Eiman, H. A., Rahman, E., Elhadi, M. A., and Nizar, S. 2014. Standardization of roselle (*Hibiscus Sabdariffa* L.) calyx cultivated in Sudan. *Journal of Medicinal Plants Research* 8.4: 217–222.

Ekins, S., Ring, B. J., Grace, J., McRobie-Belle, D. J., and Wrighton, S. A. 2000. Present and future in vitro approaches for drug metabolism. *Journal of Pharmacological And Toxicological Methods* 44.1: 313–324.

El-Saadany, S. S., Sitohy, M. Z., Labib, S. M., and El-Massry, R. A. 1991. Biochemical dynamics and hypocholesterolemic action of *Hibiscus sabdariffa* (Karkade). *Food/Nahrung* 35.6: 567–576.

Ernst, E. 2000. Adverse effects of herbal drugs in dermatology. *British Journal of Dermatology* 143.5: 923–929.

Essa, M. M., Subramanian, P., Suthakar, G., Manivasagam, T., Dakshayani, K. B., Sivaperumal, R., Subash, S., and Vinothini, G. 2006. Influence of *Hibiscus sabdariffa* (Gongura) on the levels of circulatory lipid peroxidation products and liver marker enzymes in experimental hyperammonemia. *Journal of Applied Biomedicine* 4: 53–58.

Estudante, M., Morais, J. G., Soveral, G., and Benet, L. Z. 2013. Intestinal drug transporters: An overview. *Advanced Drug Delivery Reviews* 65.10: 1340–1356.

European Medicines Agency. 2014. Guideline on bioanalytical method validation. Committee for Medicinal Products for Human Use. Retrived Aug. 19, 2016, from http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/08/WC500109686.pdf.

Evans, W. C. 2009. *Trease and Evans' pharmacognosy*. 16th Edition. USA. Elsevier Health Sciences. 169-477.

Facciolá, G., Hidestrand, M., von Bahr, C., and Tybring, G. 2001. Cytochrome P 450 isoforms involved in melatonin metabolism in human liver microsomes. *European Journal of Clinical Pharmacology* 56.12: 881–888.

Fakeye, T. 2008. Toxicity and Immunomodulatory Activity of Fractions of *Hibiscus Sabdariffa* Linn (Family Malvaceae) in Animal Models. *African Journal of Traditional, Complementary, and Alternative Medicines* 5.4: 394–398.

Fakeye, T. O., Adegoke, A. O., Omoyeni, O. C., and Famakinde, A. A. 2007a. Effect of water extract of *Hibiscus sabdariffa*, Linn (Malvaceae) – "Roselle" on excretion of a diclofenac formulation. *Phytotherapy Research* 21.1: 96–98.

Fakeye, T. O., Adisa, R., and Musa, I. E. 2009. Attitude and use of herbal medicines among pregnant women in Nigeria. *BMC Complementary and Alternative Medicine* 9.1: 53.

Fakeye, T. O., Oladipupo, T., Showande, O., and Ogunremi, Y. 2007b. Effects of coadministration of extract of *Carica papaya* Linn (family Cariaceae) on activity of two oral hypoglycemic agents. *Tropical Journal of Pharmaceutical Research* 6.1: 671–678.

Fakeye, T. O., Pal, A., and Khanuja, S. P. 2008a. Anxiolytic and sedative effects of extracts of *Hibiscus sabdariffa* Linn (family Malvaceae). *African Journal Of Medicine And Medical Sciences* 37.1: 49–54.

Fakeye, T. O., Tijani, A., and Adebisi, O. 2008b. A survey of the use of herbs among patients attending secondary-level health care facilities in Southwestern Nigeria. *Journal of Herbal Pharmacotherapy* 7.3–4: 213–227.

Fang, J., Coutts, R. T., McKenna, K. F., and Baker, G. B. 1998. Elucidation of individual cytochrome P450 enzymes involved in the metabolism of clozapine. *Naunyn-Schmiedeberg's Archives of Pharmacology* 358.5: 592–599.

Faraji, M. H., and Tarkhani, A. H. 1999. The effect of sour tea (*Hibiscus sabdariffa*) on essential hypertension. *Journal of Ethnopharmacology* 65.3: 231–236.

Farombi, E. O., and Fakoya, A. 2005. Free radical scavenging and antigenotoxic activities of natural phenolic compounds in dried flowers of *Hibiscus sabdariffa* L. *Molecular Nutrition and Food Research* 49.12: 1120–1128.

Faucette, S. R., Hawke, R. L., Lecluyse, E. L., Shord, S. S., Yan, B., Laethem, R. M., and Lindley, C, M. 2000. Validation of bupropion hydroxylation as a selective marker of human cytochrome P450 2B6 catalytic activity. *Drug Metabolism and Disposition* 28.10: 1222–1230.

Ferrero, J. L., and Brendel, K. 1997. Drug Metabolism. *Drug-Drug Interactions: Scientific and Regulatory Perspectives* 43: 131.

Finch, R. G., Greenwood, D., Whitley, R. J., and Norrby, S. R. 2010. Antibiotic and Chemotherapy. USA. Elsevier Health Sciences. 207-220. Retrieved Aug. 20, 2016, from https://books.google.com.ng/books?id=DE4Mxc3aesEC&pg=PA841&dq=Finch,+R.+G.,+Greenwood,+D.,+Whitley,+R.+J.,+and+Norrby,+S.+R.+2010.+Antibiotic+and+Chemotherapy&hl=en&sa=X&ved=0ahUKEwi6ytj7k8rQAhVIDMAKHXY9BT4Q6AEISDAH#v=onepage&q=renal%20tubular%20secretion%20of%20penicillins&f=false

Fisher, R. S., Sher, D. J., Donahue, D., Knight, L. C., Maurer, A., Urbain, J. L., and Krevsky, B. 1997. Regional differences in gastric acidity and antacid distribution: is a single pH electrode sufficient? *The American Journal of Gastroenterology* 92.2: 263–270.

Food and Drug Administration. 2012. Guidance for Industry Drug Interaction Studies — Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations *Clinical Pharmacology*. Retrieved Aug. 14, 2016,. From http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm292362.pdf.

Friedewald, W. T., Levy, R. I., and Fredrickson, D. S. 1972. Estimation of the Concentration of Low-Density Lipoprotein Cholesterol in Plasma, Without Use of the Preparative Ultracentrifuge. *Clinical Chemistry* 18.6: 499–502.

Fujita, K. 2004. Food-drug interactions via human cytochrome P450 3A (CYP3A). *Drug Metabolism and Drug Interactions* 20.4: 195–218.

Galetin, A., Clarke, S. E., and Houston, J. B. 2003. Multisite kinetic analysis of interactions between prototypical CYP3A4 subgroup substrates: midazolam, testosterone, and nifedipine. *Drug Metabolism and Disposition* 31.9: 1108–1116.

Gardiner, P., Graham, R. E., Legedza, A. T., Eisenberg, D. M., and Phillips, R. S. 2006. Factors associated with dietary supplement use among prescription medication users. *Archives of Internal Medicine* 166.18: 1968–1974.

Gautam, R. D. 2004. Sorrel–A lesser-known source of medicinal soft drink and food in India. *Natural Product Radiance* 3.5: 338–342.

George, C. F. and Shand, D. G. 2013. Presystemic Drug Elimination: Butterworths International Medical Reviews: Clinical Pharmacology and Therapeutics I. USA. Elsevier. 189-194. Retrieved Nov. 27, 2016 from https://books.google.com.ng/books?id=2QIlBQAAQBAJ&pg=PP2&dq=Presystemic+Drug+Elimination:+Butterworths+International+Medical+Reviews&hl=en&sa=X&ved=0ahUKEwjDg565lcrQAhVKKsAKHegvBHUQ6AEIGzAA#v=onepage&q=methods%20for%20studying%20first%20pass%20metabolism&f=false

Gilbert, M. R., Gonzalez, J., Hunter, K., Hess, K., Giglio, P., Chang, E., Puduvalli, V., Groves, M, D., Colman, H., Conrad, C., Levin, V., Woo, S., Mahajan, A., de Groot, J., and Alfred-Yung, W. K. 2010. A phase I factorial design study of dose-dense temozolomide alone and in combination with thalidomide, isotretinoin, and/or celecoxib as postchemoradiation adjuvant therapy for newly diagnosed glioblastoma. *Neuro-Oncology* 12.11: 1167–1172.

Goldstein, L. H., Elias, M., Ron-Avraham, G., Biniaurishvili, B. Z., Madjar, M., Kamargash, I., Braunstein, R., Berkovitch, M., and Golik, A. 2007. Consumption of herbal remedies and dietary supplements amongst patients hospitalized in medical wards. *British Journal of Clinical Pharmacology* 64.3: 373–380.

Gomez-Lechon, M. J., Donato, M. T., Castell, J. V., and Jover, R. 2004. Human hepatocytes in primary culture: the choice to investigate drug metabolism in man. *Current Drug Metabolism* 5.5: 443–462.

Gonzalez, F. J. 2005. Role of cytochromes P450 in chemical toxicity and oxidative stress: studies with CYP2E1. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* 569.1: 101–110.

Gonzalez, F. J., and Yu, A.-M. 2006. Cytochrome P450 and xenobiotic receptor humanized mice. *Annual Review of Pharmacology and Toxicology* 46: 41-64.

Gorski, J. C., Huang, S.-M., Pinto, A., Hamman, M. A., Hilligoss, J. K., Zaheer, N. A., Desai, M., Miller, M., and Hall, S. D. 2004. The effect of echinacea (*Echinacea purpurea* root) on cytochrome P450 activity *in vivo*. *Clinical Pharmacology and Therapeutics* 75.1: 89–100.

Grahame-Smith, D. G. 2016. Preclinical Psychopharmacology. USA. Elsevier. 126. Retrieved Nov. 27, 2016, from https://books.google.com.ng/books?id=YRglBQAAQBAJ&pg=PR11&dq=Preclinical+Psychopharmacology.&hl=en&sa=X&ved=0ahUKEwihrp7cl8rQAhUEB8AKHSx5ApMQ6AEIGzAA#v=onepage&q=tranquilizing%20potential%20of%20diazepam&f=false

Granfors, M. T., Backman, J. T., Laitila, J., and Neuvonen, P. J. 2004. Tizanidine is mainly metabolized by cytochrome p450 1A2 *in vitro*. *British Journal of Clinical Pharmacology* 57.3: 349–353.

Granfors, M. T., Backman, J. T., Neuvonen, M., Ahonen, J., and Neuvonen, P. J. 2004. Fluvoxamine drastically increases concentrations and effects of tizanidine: a potentially hazardous interaction. *Clinical Pharmacology and Therapeutics* 75.4: 331–341.

Guengerich, F. P. 1990. Mechanism-based inactivation of human liver microsomal cytochrome P-450 IIIA4 by gestodene. *Chemical Research in Toxicology* 3.4: 363–371.

Guengerich, F. P. 2003. Cytochromes P450, drugs, and diseases. *Molecular interventions*, 3.4: 194-204**.**

Guengerich, F. P. 2007. Cytochrome P450 and chemical toxicology. *Chemical Research in Toxicology* 21.1: 70–83.

Gugler, R., and Allgayer, H. 1990. Effects of antacids on the clinical pharmacokinetics of drugs. An update. *Clinical Pharmacokinetics* 18.3: 210–219.

Guillouzo, A. 1995. Acquisition and use of human *in vitro* liver preparations. *Cell Biology and Toxicology* 11.3–4: 141–145.

Gurley, B. J. 2012. Pharmacokinetic herb-drug interactions (part 1): origins, mechanisms, and the impact of botanical dietary supplements. *Planta Medica* 78.13: 1478–1489.

Gurrola-Díaz, C. M., García-López, P. M., Sánchez-Enríquez, S., Troyo-Sanromán, R., Andrade-González, I., and Gómez-Leyva, J. F. 2010. Effects of *Hibiscus sabdariffa* extract powder and preventive treatment (diet) on the lipid profiles of patients with metabolic syndrome (MeSy). *Phytomedicine* 17.7: 500–505.

Gwaza, L., Wolfe, A. R., Benet, L. Z., Guglielmo, B. J., Chagwedera, T. E., Maponga, C. C., Masimirembwa, C. M. 2009. *In vitro* inhibitory effects of *Hypoxis obtusa* and *Dicoma anomala* on CYP450 enzymes and p-glycoprotein. *African Journal of Pharmacy and Pharmacology* 3.11: 539–546.

Hakkola, J., Raunio, H., Purkunen, R., Saarikoski, S., Vähäkangas, K., Pelkonen, O., Edwards, R, J., Boobis, A. R., and Pasanen, M. 2001. Cytochrome P450 3A4 expression in the human fetal liver: evidence that CYP3A5 is expressed in only a limited number of fetal livers. *Neonatology* 80.3: 193–201.

Hansen, E. H., Holstein, B. E., Due, P., and Currie, C. E. 2003. International Survey of Self-Reported Medicine Use Among Adolescents. *The Annals of Pharmacotherapy* 37.3: 361–366.

Hargreaves, M. B., Jones, B. C., Smith, D. A., and Gescher, A. 1994. Inhibition of p-nitrophenol hydroxylase in rat liver microsomes by small aromatic and heterocyclic molecules. *Drug Metabolism and Disposition* 22.5: 806–810.

He, N., and Edeki, T. 2004. The inhibitory effects of herbal components on CYP2C9 and CYP3A4 catalytic activities in human liver microsomes. *American journal of therapeutics* 11.3: 206–212.

Heimark, L. D., Wienkers, L., Kunze, K., Gibaldi, M., Eddy, A. C., Trager, W. F., O'Reilly, R. A., and Goulart, D. A. 1992. The mechanism of the interaction between amiodarone and warfarin in humans. *Clinical Pharmacology and Therapeutics* 51.4: 398–407.

Henderson, M. C., Miranda, C. L., Stevens, J. F., Deinzer, M. L., and Buhler, D. R. 2000. *In vitro* inhibition of human P450 enzymes by prenylated flavonoids from hops, Humulus lupulus. *Xenobiotica* 30.3: 235–251.

Herrera-Arellano, A., Flores-Romero, S., Chavez-Soto, M. A., and Tortoriello, J. 2004. Effectiveness and tolerability of a standardized extract from *Hibiscus sabdariffa* in patients with mild to moderate hypertension: a controlled and randomized clinical trial. *Phytomedicine* 11.5: 375–382.

Hesse, L. M., von Moltke, L. L., Shader, R. I., and Greenblatt, D. J. 2001. Ritonavir, efavirenz, and nelfinavir inhibit CYP2B6 activity *in vitro*: potential drug interactions with bupropion. *Drug Metabolism and Disposition* 29.2: 100–102.

Hijazi, Y., and Boulieu, R. 2002. Contribution of CYP3A4, CYP2B6, and CYP2C9 Isoforms toN-Demethylation of Ketamine in Human Liver Microsomes. *Drug Metabolism and Disposition* 30.7: 853–858.

Hillgren, K. M., Keppler, D., Zur, A. A., Giacomini, K. M., Stieger, B., Cass, C. E., Zhang, L. and International Transporter Consortium. 2013. Emerging transporters of clinical importance: An update from the international transporter consortium. *Clinical Pharmacology and Therapeutics* 94.1: 52–63.

Hirunpanich, V., Utaipat, A., Morales, N. P., Bunyapraphatsara, N., Sato, H., Herunsale, A., et al. 2006. Hypocholesterolemic and antioxidant effects of aqueous extracts

from the dried calyx of *Hibiscus sabdariffa* L. in hypercholesterolemic rats. *Journal of Ethnopharmacology* 103.2: 252–260.

Holst, L., Wright, D., Nordeng, H., and Haavik, S. 2009. Use of herbal preparations during pregnancy: focus group discussion among expectant mothers attending a hospital antenatal clinic in Norwich, UK. *Complementary Therapies in Clinical Practice* 15.4: 225–229.

Holt, G. A., and Chandra, A. 2002. Herbs in the Modern Healthcare Environment—an Overview of Uses, Legalities, and the Role of the Healthcare Professional. *Clinical Research and Regulatory Affairs* 19.1: 83–107.

Hopkins, A. L., Lamm, M. G., Funk, J. L., and Ritenbaugh, C. 2013. *Hibiscus sabdariffa* L. in the treatment of hypertension and hyperlipidemia: A comprehensive review of animal and human studies. *Fitoterapia* 85: 84–94.

http://davesgarden.com/guides/pf/showimage/296747/

https://wormandflowers.wordpress.com/tag/gongura-plant/

Hu, Z., Yang, X., Ho, P. C. L., Chan, S. Y., Heng, P. W. S., Chan, E., Duan, W., Koh, H. L., and Zhou, Set. 2005. Herb-drug interactions. *Drugs* 65.9: 1239–1282.

Huang, S. M., and Lesko, L. J. 2004. Drug-Drug, Drug—Dietary Supplement, and Drug—Citrus Fruit and Other Food Interactions: What Have We Learned? *The Journal of Clinical Pharmacology* 44.6: 559–569.

Huang, Z., Roy, P., and Waxman, D. J. 2000. Role of human liver microsomal CYP3A4 and CYP2B6 in catalyzing N-dechloroethylation of cyclophosphamide and ifosfamide. *Biochemical pharmacology* 59.8: 961–972.

Hukkanen, J., Jacob, P., and Benowitz, N. L. 2005. Metabolism and disposition kinetics of nicotine. *Pharmacological reviews* 57.1: 79–115.

Hukkanen, J., Lassila, A., Paivarinta, K., Valanne, S., Sarpo, S., Hakkola, J., Pelkonen, O., Raunio, H. 2000. Induction and regulation of xenobiotic-metabolizing

cytochrome P450s in the human A549 lung adenocarcinoma cell line. *American Journal of Respiratory Cell and Molecular Biology* 22.3: 360–366.

Ingelman-Sundberg, M. 2004. Pharmacogenetics of cytochrome P450 and its applications in drug therapy: the past, present and future. *Trends in pharmacological sciences* 25.4: 193–200.

Ingelman-Sundberg, M. 2005. Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity. *The Pharmacogenomics Journal* 5.1: 6–13.

Ioannides, C. 2002. Pharmacokinetic interactions between herbal remedies and medicinal drugs. *Xenobiotica* 32.6: 451–478.

Izzo, A. A. 2005. Herb–drug interactions: an overview of the clinical evidence. *Fundamental and Clinical Pharmacology* 19.1: 1–16.

Izzo, A. A., and Ernst, E. 2009. Interactions between herbal medicines and prescribed drugs. *Drugs* 69.13: 1777–1798.

Izzo, A. A., and Ernst, E. 2012. Interactions between herbal medicines and prescribed drugs. *Drugs* 61.15: 2163–2175.

Jackman, R. L., and Smith, J. L. 1996. Anthocyanins and betalains. In Hendry, G, A, F., and Houghton, J, D. (Eds.), *Natural Food Colorants*: 244–309. Springer US. Retrieved Feb 24, 2016, from .http://link.springer.com/chapter/10.1007/978-1-4615-2155-6_8,

Jiang, X., Williams, K. M., Liauw, W. S., Ammit, A. J., Roufogalis, B. D., Duke, C. C., Day, R, O., and McLachlan, A. J. 2004. Effect of St John's wort and ginseng on the pharmacokinetics and pharmacodynamics of warfarin in healthy subjects. *British Journal of Clinical Pharmacology* 57.5: 592–599.

Jones, M. T. E. 1997. The use of other drugs to allow a lower dosage of cyclosporin to be used. *Clinical Pharmacokinetics* 32.5: 357–367.

Juurlink, D. N., Mamdani, M., Kopp, A., Laupacis, A., and Redelmeier, D. A. 2003. Drug-drug interactions among elderly patients hospitalized for drug toxicity. *JAMA: Journal of the American Medical Association* 289.13: 1652–1658.

Kalow,W. 2001. Interethnic differences in drug response. *Drugs and the Pharmaceutical Sciences* 113: 109–134.

Kamath, A. V., Morrison, R. A., Mathias, N. R., Dando, S. A., Marino, A. M., and Chong, S. 2007. Modulation of tight junctions does not predict oral absorption of hydrophilic compounds: Use of caco-2 and calu-3 cells. *Archives of Pharmacological Research* 30.8: 1002–1007.

Kanda, Y., Kami, M., Matsuyama, T., Mitani, K., Chiba, S., Yazaki, Y., Hirai, H. 1998. Plasma concentration of itraconazole in patients receiving chemotherapy for hematological malignancies: the effect of famotidine on the absorption of itraconazole. *Hematological Oncology* 16.1: 33–37.

Kantola, T., Kivistö, K. T., and Neuvonen, P. J. 1998. Erythromycin and verapamil considerably increase serum simvastatin and simvastatin acid concentrations*. *Clinical Pharmacology and Therapeutics* 64.2: 177–182.

Karyekar, C. S., Eddington, N. D., and Dowling, T. C. 2002. Effect of St. John's Wort extract on intestinal expression of cytochrome P4501A2: studies in LS180 cells. *Journal of Postgraduate Medicine* 48.2: 97–100.

Katz, P. O., Gerson, L. B. and Vela, M. F. 2013. Guidelines for the diagnosis and management of gastroesophageal reflux disease. *The American Journal of Gastroenterology* 108.3: 308–328.

Katzung, B. G., Masters, S. B., and Trevor, A. J. 2009. *Basic and Clinical Pharmacology, 11th Edition*. McGraw Hill Professional. 1137-1145. Retrieved Nov. 27, 2016, from https://books.google.com.ng/books?id=4O7ghcthkt4C&dq=Basic+and+Clinical+

Pharmacology%2C+11th+Edition.&focus=searchwithinvolume&q=important+drug+interactions+and+their+mechanisms

Kaufman, D. W., Kelly, J. P., Rosenberg, L., Anderson, T. E., and Mitchell, A. A. 2002. Recent patterns of medication use in the ambulatory adult population of the United States: the Slone survey. *Journal of America Medical Association* 287.3: 337–344.

Kelly, W. N. 2001. Potential risks and prevention, Part 4: Reports of significant adverse drug events. *American Journal of Health-System Pharmacy* 58.15: 1406–1412.

Kempf, D. J., Marsh, K. C., Kumar, G., Rodrigues, A. D., Denissen, J. F., McDonald, E., Kukulka, M. J., Hsu, A., Granneman, G. R., Baroldi, P. A., Sun, E., Pizzuti, D., Plattner, J. J., Norbeck, D. W., and Leonard, J. M. 1997. Pharmacokinetic enhancement of inhibitors of the human immunodeficiency virus protease by coadministration with ritonavir. *Antimicrobial Agents and Chemotherapy* 41.3: 654–660.

Kennedy, J., Wang, C. C., and Wu, C. H. 2008. Patient Disclosure about Herb and Supplement Use among Adults in the US. *Evidence-Based Complementary and Alternative Medicine* 5.4: 451–456.

Kent, U. M., Jushchhyshyn, M. I., and Hollenberg, P. F. 2001. Mechanism-based inactivators as probes of cytochrome P450 structure and function. *Current Drug Metabolism* 2.3: 215–243.

Keogh, A., Spratt, P., McCosker, C., Macdonald, P., Mundy, J., and Kaan, A. 1995. Ketoconazole to reduce the need for cyclosporine after cardiac transplantation. *New England Journal of Medicine* 333.10: 628–634.

Kharasch, E. D., Thummel, K. E., Mhyre, J., and Lillibridge, J. H. 1993. Single-dose disulfiram inhibition of chlorzoxazone metabolism: a clinical probe for P450 2E1. *Clinical Pharmacology and Therapeutics* 53.6: 643–650.

Kim, B. C., Ban, E., Park, J. S., Song, Y. K., and Kim, C. K. 2005. Determination of simvastatin in human plasma by column-switching HPLC with UV detection. *Journal of Liquid Chromatography and Related Technologies* 27.19: 3089–3102.

Kim, H. J., Chun, Y. J., Park, J. D., Kim, S. I., Roh, J. K., and Jeong, T. C. 1997. Protection of rat liver microsomes against carbon tetrachloride-induced lipid peroxidation by red ginseng saponin through cytochrome P450 inhibition. *Planta Medica* 63.5: 415–418.

Kim, K. A., Park, J. Y., Lee, J. S., and Lim, S. 2003. Cytochrome P450 2C8 and CYP3A4/5 are involved in chloroquine metabolism in human liver microsomes. *Archives of Pharmacological Research* 26.8: 631–637.

Kimura, Y., Ito, H., Ohnishi, R., and Hatano, T. 2010. Inhibitory effects of polyphenols on human cytochrome P450 3A4 and 2C9 activity. *Food and Chemical Toxicology* 48.1: 429–435.

Kirchheiner, J., and Brockmöller, J. 2005. Clinical consequences of cytochrome P450 2C9 polymorphisms. *Clinical Pharmacology and Therapeutics* 77.1: 1–16.

Kitada, M., and Kamataki, T. 1994. Cytochrome P450 in human fetal liver: significance and fetal-specific expresson. *Drug Metabolism Reviews* 26.1–2: 305–323.

Klaassen, C. D., Amdur, M., and Doull, J. 2013. Casarett and Doull's toxicology: the basic science of poisons. McGraw-Hill New York (NY). Retrieved Feb. 24, 2016, from https://www.researchgate.net/profile/John_Doull/publication/44361322_Toxicology__the_basic_science_of_poisons__Casarett_and_Doull/links/00b7d52297092af945000000.pdf.

Knights, K. M., Rowland, A. and Miners, J O. 2013. Renal drug metabolism in humans: the potential for drug–endobiotic interactions involving cytochrome P450 (CYP) and UDP-Glucuronosyltransferase (UGT). *British Journal of Clinical Pharmacology* 76.4: 587–602.

Kolawole, J. A., and Maduenyi, A. 2004. Effect of *Zobo* drink (*Hibiscus sabdariffa* water extract) on the pharmacokinetics of acetaminophen in human volunteers. *European Journal of Drug Metabolism and Pharmacokinetics* 29.1: 25–29.

Komatsu, T., Yamazaki, H., Asahi, S., Gillam, E. M., Guengerich, F. P., Nakajima, M., and Yokol, T. 2000. Formation of a dihydroxy metabolite of phenytoin in human liver microsomes/cytosol: roles of cytochromes P450 2C9, 2C19, and 3A4. *Drug Metabolism and Disposition* 28.11: 1361–1368.

Kong, W. M., Chik, Z., Ramachandra, M., Subramaniam, U., Aziddin, R. E. R., and Mohamed, Z. 2011. Evaluation of the effects of *Mitragyna speciosa* alkaloid extract on cytochrome P450 enzymes using a high throughput assay. *Molecules* 16.9: 7344–7356.

König, J., Müller, F., and Fromm, M. F. 2013. Transporters and drug-drug interactions: important determinants of drug disposition and effects. *Pharmacological Reviews* 65.3: 944–966.

Krejcie, R. V., and Morgan, D. W. 1970. Determining sample size for research activities. *Educational and Psychological Measurement* Retrieved Mar. 20, 2013, from http://www.eric.ed.gov/ERICWebPortal/detail?accno=EJ026025.

Kremers, P. 2002. *In vitro* tests for predicting drug-drug interactions: The need for validated procedures. *Pharmacology and Toxicology* 91.5: 209–217.

Kristensen, M. B. 1976. Drug interactions and clinical pharmacokinetics. *Clinical Pharmacokinetics* 1.5: 351–372.

Kunze, K. L., and Trager, W. F. 1993. Isoform-selective mechanism-based inhibition of human cytochrome P450 1A2 by furafylline. *Chemical Research in Toxicology* 6.5: 649–656.

Kuriyan, R., Kumar, D. R., R, R., and Kurpad, A. V. 2010. An evaluation of the hypolipidemic effect of an extract of *Hibiscus Sabdariffa* leaves in hyperlipidemic

Indians: a double blind, placebo controlled trial. *BMC Complementary and Alternative Medicine* 10.1: 27.

Lang, T., Klein, K., Fischer, J., Nüssler, A. K., Neuhaus, P., Hofmann, U., Eichelbaum, M., Schwab, M., and Zanger, U. M. 2001. Extensive genetic polymorphism in the human CYP2B6 gene with impact on expression and function in human liver. *Pharmacogenetics and Genomics* 11.5: 399–415.

Langowski, J., and Long, A. 2002. Computer systems for the prediction of xenobiotic metabolism. *Advanced Drug Delivery Reviews* 54.3: 407–415.

Larsen, J. T., and Brøsen, K. 2005. Consumption of charcoal-broiled meat as an experimental tool for discerning CYP1A2-mediated drug metabolism *in vivo*. *Basic and Clinical Pharmacology and Toxicology* 97.3: 141–148.

Lasser, K. E., Allen, P. D., Woolhandler, S. J., Himmelstein, D. U., Wolfe, S. M., and Bor, D. H. 2002. Timing of new black box warnings and withdrawals for prescription medications. *Journal of the American Medical Association* 287.17: 2215–2220.

Lazarou, J., Pomeranz, B. H., and Corey, P. N. 1998. Incidence of adverse drug reactions in hospitalized patients. *Journal of the American Medical Association*, 279.15: 1200–1205.

Leape, L. L., Brennan, T. A., Laird, N., Lawthers, A. G., Localio, A. R., Barnes, B. A., Hebert, L., Newhouse, J. P., Weiler, P. C., and Hiatt, H. 1991. The nature of adverse events in hospitalized patients: results of the Harvard Medical Practice Study II. *New England Journal of Medicine* 324.6: 377–384.

Lee, W., and Kim, R. B. 2004. Transporters and Renal Drug Elimination. *Annual Review of Pharmacology and Toxicology* 44.1: 137–166.

Lewis, D. F. 2003. Human cytochromes P450 associated with the phase 1 metabolism of drugs and other xenobiotics: a compilation of substrates and inhibitors of the

CYP1, CYP2 and CYP3 families. *Current Medicinal Chemistry* 10.19: 1955–1972.

Lewis, D. F. 2004. 57 varieties: the human cytochromes P450. *Pharmacogenomics* 5(3): 305–318.

Lilja, J. J., Kivistö, K. T., and Neuvonen, P. J. 1999. Grapefruit juice increases serum concentrations of atorvastatin and has no effect on pravastatin. *Clinical Pharmacology and Therapeutics* 66.2: 118–127.

Lilja, J. J., Kivistö, K. T., and Neuvonen, P. J. 2000. Duration of effect of grapefruit juice on the pharmacokinetics of the CYP3A4 substrate simvastatin. *Clinical Pharmacology and Therapeutics* 68.4: 384–390.

Lilja, J. J., Neuvonen, M., and Neuvonen, P. J. 2004. Effects of regular consumption of grapefruit juice on the pharmacokinetics of simvastatin. *British Journal of Clinical Pharmacology* 58.1: 56–60.

Liljefors, T., Krogsgaard-Larsen, P., and Madsen, U. 2002. Textbook of drug design and discovery. CRC Press. 342-358. Retrieved Feb. 23, 2016, from https://books.google.com.ng/books?id=EL-UI6t8omQC&pg=PA271&dq=Textbook+of+drug+design+and+discovery.+Liljefors,+T.,+Krogsgaard-Larsen,+P.,+and+Madsen,+U.+2002.+T&hl=en&sa=X&ved=0ahUKEwjM4c7iocrQAhXEDcAKHc1EA7cQ6AEIGTAA#v=onepage&q=noncompetitive%20inhibition&f=false

Lin, T. L., Lin, H. H., Chen, C. C., Lin, M. C., Chou, M. C., and Wang, C. J. 2007. *Hibiscus sabdariffa* extract reduces serum cholesterol in men and women. *Nutrition Research* 27.3: 140–145.

Lomaestro, B. M., and Bailie, G. R. 1991. Quinolone-cation interactions: a review. *DICP: The Annals of Pharmacotherapy* 25.11: 1249–1258.

Luo, G., Guenthner, T., Gan, L.-S., and Humphreys, W. G. 2004. CYP3A4 induction by xenobiotics: biochemistry, experimental methods and impact on drug discovery and development. *Current Drug Metabolism* 5.6: 483–505.

Madsen, H., Andersen, S., Nielsen, R. G., Dolmer, B. S., Høst, A., and Damkier, A. 2003. Use of complementary/alternative medicine among paediatric patients. *European Journal of Pediatrics* 162.5: 334–341.

Maganha, E. G., Halmenschlager, R. da C., Rosa, R. M., Henriques, J. A. P., Ramos, A. L. L. de P., and Saffi, J. 2010. Pharmacological evidences for the extracts and secondary metabolites from plants of the genus Hibiscus. *Food Chemistry* 118.1: 1–10.

Mahadevan, N., Shivali, K. P., and Kamboj, P. 2009b. *Hibiscus sabdariffa* Linn.-an overview. *Natural Product Radiance* 8.1: 77–83.

Mahmoud, B. M. 1994. Significant reduction in chloroquine bioavailablity following coadministration with the Sudanese beverages Aradaib, Karkadi and Lemon. *Journal of Antimicrobial Chemotherapy* 33.5: 1005–1009.

Martignoni, M., Groothuis, G. M., and de Kanter, R. 2006. Species differences between mouse, rat, dog, monkey and human CYP-mediated drug metabolism, inhibition and induction. *Expert Opinion on Drug Metabolism and Toxicology* 2.6: 875–894.

McDonnell, P. J., and Jacobs, M. R. 2002. Hospital admissions resulting from preventable adverse drug reactions. *Annals of Pharmacotherapy* 36.9: 1331–1336.

McGinnity, D. F., and Riley, R. J. 2001. Predicting drug pharmacokinetics in humans from in vitro metabolism studies. *Biochemical Society Transactions* 29.2: 135–139.

McKay, D. L., Chen, C. O., Saltzman, E., and Blumberg, J. B. 2010. *Hibiscus sabdariffa* L. tea (tisane) lowers blood pressure in prehypertensive and mildly hypertensive adults. *The Journal of Nutrition* 140.2: 298–303.

Miller, M. F., Bellizzi, K. M., Sufian, M., Ambs, A. H., Goldstein, M. S., and Ballard-Barbash, R. 2008. Dietary supplement use in individuals living with cancer and other chronic conditions: a population-based study. *Journal of the American Dietetic Association* 108.3: 483–494.

Möhrke, W., Knauf, H. and Mutschler, E. 1997. Pharmacokinetics and pharmacodynamics of triamterene and hydrochlorothiazide and their combination in healthy volunteers. *International Journal of Clinical Pharmacology and Therapeutics 3*5.10: 447–452.

Mohutsky, M. A., Anderson, G. D., Miller, J. W., and Elmer, G. W. 2006. *Ginkgo biloba*: evaluation of CYP2C9 drug interactions *in vitro* and *in vivo*. *American Journal of Therapeutics* 13.1: 24–31.

Murray, S., Lake, B. G., Gray, S., Edwards, A. J., Springall, C., Bowey, E. A., Williamson, G., Boobis, A. R., and Gooderham, N. J. 2001. Effect of cruciferous vegetable consumption on heterocyclic aromatic amine metabolism in man. *Carcinogenesis* 22.9: 1413–1420.

National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). 2002. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation* 106.25: 3143–3421.

Nebert, D. W., and Russell, D. W. 2002. Clinical importance of the cytochromes P450. *The Lancet* 360.9340: 1155–1162.

Nelson, D. R., Zeldin, D. C., Hoffman, S. M., Maltais, L. J., Wain, H. M., and Nebert, D. W. 2004. Comparison of cytochrome P450 (CYP) genes from the mouse and human genomes, including nomenclature recommendations for genes, pseudogenes and alternative-splice variants. *Pharmacogenetics and Genomics* 14.1: 1–18.

Neuhofel, A. L., Wilton, J. H., Victory, J. M., Hejmanowski, L. G., and Amsden, G. W. 2002. Lack of bioequivalence of ciprofloxacin when administered with calcium-fortified orange juice: A new twist on an old interaction. *The Journal of Clinical Pharmacology* 42.4: 461–466.

Niemi, M. 2010. Transporter pharmacogenetics and statin toxicity. *Clinical Pharmacology and Therapeutics* 87.1: 130–133.

Niemi, M., Leathart, J. B., Neuvonen, M., Backman, J. T., Daly, A. K., and Neuvonen, P. J. 2003. Polymorphism in CYP2C8 is associated with reduced plasma concentrations of repaglinide. *Clinical Pharmacology and Therapeutics* 74.4: 380–387.

Niwa, T., Shiraga, T., and Takagi, A. 2005. Effect of antifungal drugs on cytochrome P450 (CYP) 2C9, CYP2C19, and CYP3A4 activities in human liver microsomes. *Biological and Pharmaceutical Bulletin* 28.9: 1805–1808.

Nordeng, H., Bayne, K., Havnen, G. C., and Paulsen, B. S. 2011. Use of herbal drugs during pregnancy among 600 Norwegian women in relation to concurrent use of conventional drugs and pregnancy outcome. *Complementary Therapies in Clinical Practice* 17.3: 147–151.

Obach, R. S., Walsky, R. L., Venkatakrishnan, K., Gaman, E. A., Houston, J. B., and Tremaine, L. M. 2006. The utility of *in vitro* cytochrome P450 inhibition data in the prediction of drug-drug interactions. *Journal of Pharmacology and Experimental Therapeutics* 316.1: 336–348.

Obermeier, M. T., White, R. E., and Yang, C. S. 1995. Effects of bioflavonoids on hepatic P450 activities. *Xenobiotica* 25.6: 575–584.

Oboh, G., and Elusiyan, C. A. 2004. Nutrient composition and antimicrobial activity of sorrel drinks (soborodo). *Journal of Medicinal Food* 7.3: 340–342.

Odigie, I. P., Ettarh, R. R., and Adigun, S. A. 2003. Chronic administration of aqueous extract of *Hibiscus sabdariffa* attenuates hypertension and reverses cardiac

hypertrophy in 2K-1C hypertensive rats. *Journal of Ethnopharmacology* 86.2–3.: 181–185.

Ogawa, R., and Echizen, H. 2011. Clinically significant drug interactions with antacids: an update. *Drugs* 71.14: 1839–1864.

Ogilvie, B. W., Usuki, E., Yerino, P., and Parkinson, A. 2008. *In vitro* approaches for studying the inhibition of drug-metabolizing enzymes and identifying the drug-metabolizing enzymes responsible for the metabolism of drugs (reaction phenotyping) with emphasis on cytochrome P450. *Drugs and the Pharmaceutical Sciences* 179: 231-358.

Ogilvie, R. I., and Ruedy, J. 1967. Adverse drug reactions during hospitalization. *Canadian Medical Association Journal* 97.24: 1450–1457.

Olisa, N. S., and Oyelola, F. T. 2009. Evaluation of use of herbal medicines among ambulatory hypertensive patients attending a secondary health care facility in Nigeria. *International Journal of Pharmacy Practice* 17.2: 101–105.

Omkvist, D. H., Brodin, B., and Nielsen, C. U. 2010. Ibuprofen is a non-competitive inhibitor of the peptide transporter hPEPT1 (SLC15A1): possible interactions between hPEPT1 substrates and ibuprofen. *British Journal of Pharmacology* 161.8: 1793–1805.

Onyenekwe, P. C., Ajani, E. O., Ameh, D. A., and Gamaniel, K. S. 1999. Antihypertensive effect of roselle (*Hibiscus sabdariffa*) calyx infusion in spontaneously hypertensive rats and a comparison of its toxicity with that in Wistar rats. *Cell Biochemistry and Function* 17.3: 199–206.

Ostrovskaya, R. U. and Voronina, T. A. 1977. Antagonistic effect of bicuculline and thiosemicarbazide on the tranquilizing action of diazepam. *Bulletin of Experimental Biology and Medicine* 83.3: 332–334.

Owulade, M. O., Eghianruwa, K. I. and Daramola, F. O. 2004, Effects of aqueous extracts of *Hibiscus sabdariffa* calyces and *Ocimun gratissimum* leaves on interstinal transit in rats. *African Journal of Biomedical Research* 7.1:31-39

Pacifici, G. M., and Pelkonen, O. 2001. Interindividual variability in human drug metabolism. CRC Press. 120. Retrieved Feb. 24, 2016, from https://books.google.com/books?hl=enandlr=andid=mNKWjla41qUCandoi=fndandpg=PR9anddq=Pacifici+%26+Pelkonen+2001andots=BZexBUZKkYandsig=4hqIdB42ejqMhvpE08yE2_Kuv5U.

Paine, M. F., Hart, H. L., Ludington, S. S., Haining, R. L., Rettie, A. E., and Zeldin, D. C. 2006. The human intestinal cytochrome P450 “pie.” *Drug Metabolism and Disposition* 34.5: 880–886.

Palleria, C., Di Paolo, A., Giofrè, C., Caglioti, C., Leuzzi, G., Siniscalchi, A., De Sarro, G. 1., and Gallelli, L. 2013. Pharmacokinetic drug-drug interaction and their implication in clinical management. *Journal of Research in Medical Sciences : The Official Journal of Isfahan University of Medical Sciences* 18.7: 601–610.

Palmer, M. E., Haller, C., McKinney, P. E., Klein-Schwartz, W., Tschirgi, A., Smolinske, S. C., Woolf, A., Sprague, B. M., Ko, R., Everson, G., Nelson, L. S., Dodd-Butera, T., Bartlett, W. D., and Landzberg, B. R. 2003. Adverse events associated with dietary supplements: an observational study. *The Lancet* 361.9352: 101–106.

Parkinson, A. 2001. Biotransformation of xenobiotics. McGraw-Hill New York. Retrieved February 24, 2016, from .http://www.farmasi.unud.ac.id/ind/wp-content/uploads/Bio-Transformation-of-Xenobiotics.pdf.

Patki, K. C., von Moltke, L. L., and Greenblatt, D. J. 2003. *In vitro* metabolism of midazolam, triazolam, nifedipine, and testosterone by human liver microsomes and recombinant cytochromes P450: role of cyp3A4 and cyp3A5. *Drug Metabolism and Disposition* 31.7: 938–944.

Pelkonen, O. 2002. Human CYPs: *in vivo* and clinical aspects. *Drug metabolism reviews* 34.1–2: 37–46.

Pelkonen, O., Mäeenpäeä, J., Taavitsainen, P., Rautio, A., and Raunio, H. 1998. Inhibition and induction of human cytochrome P450 (CYP) enzymes. *Xenobiotica* 28.12: 1203–1253.

Pelkonen, O., Rautio, A., Raunio, H., and Pasanen, M. 2000. CYP2A6: a human coumarin 7-hydroxylase. *Toxicology* 144.1: 139–147.

Pelkonen, O., Turpeinen, M., Uusitalo, J., Rautio, A., and Raunio, H. 2005. Prediction of drug metabolism and interactions on the basis of *in vitro* investigations. *Basic and Clinical Pharmacology and Toxicology* 96.3: 167–175.

Peter, R., Boecker, R., Beaune, P. H., Iwasaki, M., Guengerich, F. P., and Yang, C. S. 1990. Hydroxylation of chlorzoxazone as a specific probe for human liver cytochrome P_{450}2E1. *Chemical Research in Toxicology* 3.6: 566–573.

Piscitelli, S. C., Rodvold, K. A., and Pai, M. P. 2011. Drug interactions in infectious diseases. Springer Science and Business Media.13-39. Retrieved Nov. 28, 2016, from https://books.google.com.ng/books?id=EtwFCAAAQBAJ&printsec=frontcover&dq=Drug+Interactions+in+Infectious+Diseases&hl=en&sa=X&ved=0ahUKEwjTxIbTqcrQAhUmEJoKHUCsBJgQ6AEIGjAA#v=onepage&q=Drug%20Interactions%20in%20Infectious%20Diseases&f=false

Pitarque, M., Rodríguez-Antona, C., Oscarson, M., and Ingelman-Sundberg, M. 2005. Transcriptional regulation of the human CYP2A6 gene. *Journal of Pharmacology and Experimental Therapeutics* 313.2: 814–822.

Plosker, G. L., and Scott, L. J. 2003. Saquinavir. *Drugs* 63.12: 1299–1324.

Pollock, B. G., Wylie, M., Stack, J. A., Sorisio, D. A., Thompson, D. S., Kirshner, M. A., Diane, S., Thompson, M. D., Margaret, A., Kirshner, B. A., Maggie, M. Folan, B. S. N., Kelly, A., and Condifer, B. S. 1999. Inhibition of caffeine metabolism by

estrogen replacement therapy in postmenopausal women. *The Journal of Clinical Pharmacology* 39.9: 936–940.

Prommetta, P., Phivthong-ngam, L., Chaichantipyuth, C., Niwattisaiwong, N., and Lawanprasert, S. 2006. Aqueous extract of the calyces of *Hibiscus sabdariffa* Linn: effects on hepatic cytochrome P450 and subacute toxicity in rats. *Thailand Journal of Pharmaceutical Sciences* 30.1–2: 8–18.

Prueksaritanont, T., Ma, B., and Yu, N. 2003. The human hepatic metabolism of simvastatin hydroxy acid is mediated primarily by CYP3A, and not CYP2D6. *British Journal of Clinical Pharmacology* 56.1: 120–124.

Radimer, K., Bindewald, B., Hughes, J., Ervin, B., Swanson, C., and Picciano, M. F. 2004. Dietary supplement use by US adults: data from the National Health and Nutrition Examination Survey, 1999–2000. *American Journal of Epidemiology* 160.4: 339–349.

Rau, T., Wohlleben, G., Wuttke, H., Thuerauf, N., Lunkenheimer, J., Lanczik, M. and Eschenhagen, T. 2004. CYP2D6 genotype: impact on adverse effects and nonresponse during treatment with antidepressants—a pilot study. *Clinical Pharmacology and Therapeutics* 75.5: 386–393.

Raucy, J. L., Lasker, J. M., Lieber, C. S., and Black, M. 1989. Acetaminophen activation by human liver cytochromes P_{450} 2E1 and P_{450} 1A2. *Archives of Biochemistry and Biophysics* 271.1: 270–283.

Rendic, S. 2002. Summary of information on human CYP enzymes: human P450 metabolism data. *Drug Metabolism Reviews* 34.1–2: 83–448.

Rettie, A. E. and Jones, J. P. 2005. Clinical and toxicological relevance of CYP2C9: Drug-drug interactions and pharmacogenetics. *Annual Review of Pharmacology and Toxicology* 45.1: 477–494.

Ritschel, W. A. 1992. Handbook of basic pharmacokinetics-- including clinical applications. Hamilton, IL: Drug Intelligence Publications. 61. Retrieved Nov. 28,

2016, from https://books.google.com.ng/books?id=kuRsAAAAMAAJ&dq=Handbook+of+basic+pharmacokinetics--+including+clinical+applications.&focus=searchwithinvolume&q=absorption+transport+system

Robinson, A., and McGrail, M. R. 2004. Disclosure of CAM use to medical practitioners: a review of qualitative and quantitative studies. *Complementary Therapies in Medicine* 12.2–3: 90–98.

Rodrigues, A. D. 1999. Integrated cytochrome P450 reaction phenotyping. *Biochemical Pharmacolology* 57: 465–480.

Rodrigues, A. D., and Lin, J. H. 2001. Screening of drug candidates for their drug–drug interaction potential. *Current Opinion in Chemical Biology* 5.4: 396–401.

Rodrigues, A., and Rushmore, T. H. 2002. Cytochrome P450 pharmacogenetics in drug development: *in vitro* studies and clinical consequences. *Current Drug Metabolism* 3.3: 289–309.

Rose, K. D., Croissant, P. D., Parliament, C. F., and Levin, M. B. 1990. Spontaneous spinal epidural hematoma with associated platelet dysfunction from excessive garlic ingestion: a case report. *Neurosurgery* 26.5: 880–882.

Rowland, M. 1980. Plasma protein binding and therapeutic drug monitoring. *Therapeutic Drug Monitoring* 2.1: 29–37.

Rumack, B. H. 2004. Acetaminophen misconceptions. *Hepatology* 40.1: 10–15.

Sadowski, D. C. 1994. Drug interactions with antacids. Mechanisms and clinical significance. *Drug Safety* 11.6: 395–407.

Safdar, N., Handelsman, J. and Maki, D. G (2004), Does combination antimicrobial therapy reduce mortality in gram-negative bacteraemia? A meta-analysis. *The Lancet Infectious Diseases* 4.8: 519–527.

Samali, A., Kirim, R. A. and Mustapha, K. B. 2012, Qualitative and quantitative evaluation of some herbal teas commonly consumed in Nigeria. *African Journal of Pharmacy and Pharmacology* 6.6: Retrieved Sept. 18, 2016, from http://www.academicjournals.org/AJpp/abstracts/abstracts/abstract%202012/15%20Feb/Samali%20et%20al.html.

Sand, P. G., Dreiseitel, A., Stang, M., Schreier, P., Oehme, A., Locher, S., and Hajak, G. 2010. Cytochrome P450 2C19 inhibitory activity of common berry constituents. *Phytotherapy Research* 24.2: 304–307.

Sarkar, R., Hossen, M., Howlader, S. I., Rahman, A. and Dey, A. 2012. Anti-diarrheal, analgesic and anti-microbial activities of the plant Lalmesta (*Hibiscus Sabdariffa*): A review. International Journal of Pharmaceutical and Life Sciences 1.3: Retrieved Oct. 3, 2016 from http://www.banglajol.info/bd/index.php/IJPLS/article/view/12978.

Schachter, M. 2005. Chemical, pharmacokinetic and pharmacodynamic properties of statins: an update. *Fundamental and Clinical Pharmacology* 19.1: 117–125.

Sesardic, D., Boobis, A. R., Murray, B. P., Murray, S., Segura, J., Torre, R., and Davies, D. S. 1990. Furafylline is a potent and selective inhibitor of cytochrome P450IA2 in man. *British Journal of Clinical Pharmacology* 29.6: 651–663.

Shalansky, S., Lynd, L., Richardson, K., Ingaszewski, A., and Kerr, C. 2007. Risk of Warfarin-Related Bleeding Events and Supratherapeutic International Normalized Ratios Associated with Complementary and Alternative Medicine: A Longitudinal Analysis. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy* 27.9: 1237–1247.

Sharrett, A. R., Ballantyne, C. M., Coady, S. A., Heiss, G., Sorlie, P. D., Catellier, D. 2001. Coronary heart disease prediction from lipoprotein cholesterol levels, triglycerides, lipoprotein (a), apolipoproteins AI and B, and HDL density subfractions the atherosclerosis risk in communities (ARIC) study. *Circulation* 104.10: 1108–1113.

Shen, S., Marchick, M. R., Davis, M. R., Doss, G. A., and Pohl, L. R. 1999. Metabolic activation of diclofenac by human cytochrome P450 3A4: role of 5-hydroxydiclofenac. *Chemical Research in Toxicology* 12.2: 214–222.

Shi, S., and Klotz, U. 2012. Drug interactions with herbal medicines. *Clinical Pharmacokinetics* 51.2: 77–104.

Shimada, T., and Fujii-Kuriyama, Y. 2004. Metabolic activation of polycyclic aromatic hydrocarbons to carcinogens by cytochromes P450 1A1 and1B1. *Cancer Science* 95.1: 1–6.

Shimada, T., Hayes, C. L., Yamazaki, H., Amin, S., Hecht, S. S., Guengerich, F. P., and Sutter, T. R. 1996. Activation of chemically diverse procarcinogens by human cytochrome P-450 1B1. *Cancer Research* 56.13: 2979–2984.

Smith, D. A., Di, L., and Kerns, E. H. 2010. The effect of plasma protein binding on in vivo efficacy: misconceptions in drug discovery. *Nature Reviews Drug Discovery* 9.12: 929–939.

Smith, G. B., Bend, J. R., Bedard, L. L., Reid, K. R., Petsikas, D., and Massey, T. E. 2003. Biotransformation of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in peripheral human lung microsomes. *Drug Metabolism and Disposition* 31.9: 1134–1141.

Smith, M. B., Christensen, N., Wang, S., Strohecker, J., Day, J. D., Weiss, J. P., Crandall, B. G., Osborn, J. S., Anderson, J. L., Horne, B. D., Muhlestein, J. B., Lappe, D. L., Moss, H., Oliver, J., Viau, K., and Bunch, T. J. 2010. Warfarin knowledge in patients with atrial fibrillation: implications for safety, efficacy, and education strategies. *Cardiology* 116.1: 61–69.

Solus, J. F., Arietta, B. J., Harris, J. R., Sexton, D. P., Steward, J. Q., McMunn, C., Ihrie, P., Mehall, J. M., Edwards, T. L., Dawson, E. P. 2004. Genetic variation in eleven phase I drug metabolism genes in an ethnically diverse population. *Pharmacogenomics* 5.7: 895–931.

Sørensen, J. M. 2002. Herb-drug, food-drug, nutrient-drug, and drug-drug interactions: mechanisms involved and their medical implications. *Journal of Alternative and Complementary Medicine* 8.3: 293–308.

Spigset, O., Hägg, S., Söderström, E., and Dahlqvist, R. 1999. Lack of correlation between fluvoxamine clearance and CYP1A2 activity as measured by systemic caffeine clearance. *European Journal of Clinical Pharmacology* 54.12: 943–946.

Strandell, J., Neil, A., and Carlin, G. 2004. An approach to the *in vitro* evaluation of potential for cytochrome P450 enzyme inhibition from herbals and other natural remedies. *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology* 11.2–3: 98–104.

Sugimoto, K. 2001. Different effects of St John's Wort on the pharmacokinetics of simvastatin and pravastatin. *Clinical Pharmacology and Therapeutics* 70.6: 518–524.

Sukhapat, N., Ungphaiboon, S., Itharat, A., Puripattanavong, J., and Pinsuwan, S. 2004. Influence of pH on antioxidant activity of roselle (*Hibiscus sabdariffa* L.) extract in aqueous solution. The 10th World Congress on Clinical Nutrition: Nutrition in the Next Decade: Nutraceutical/Functional Food: Product Performance in Health, Disease and Safety. *Abstract book. Organized by PSU, INC and BIOTEC*: 184.

Sullivan-Klose, T. H., Ghanayem, B. I., Bell, D. A., Zhang, Z.-Y., Kaminsky, L. S., Shenfleld, G. M. 1996. The role of the CFP2C9-Leu 359 allelic variant in the tolbutamide polymorphism. *Pharmacogenetics and Genomics* 6.4: 341–349.

Tang, W., Stearns, R. A., Kwei, G. Y., Iliff, S. A., Miller, R. R., Egan, M. A., Yu, N. X., Dean, D. C., Kumar, S., Shou, M., Lin, J. H., and Baillie, T, A. 1999. Interaction of diclofenac and quinidine in monkeys: stimulation of diclofenac metabolism. *Journal of Pharmacology and Experimental Therapeutics* 291.3: 1068–1074.

Tateno, C., Yoshizane, Y., Saito, N., Kataoka, M., Utoh, R., Yamasaki, C., Tachibana, A., Soeno, Y., Asahina, K., Hino, H., Asahara, T., Yokoi, T., Furukawa, T., and

Yoshizato, K. 2004. Near completely humanized liver in mice shows human-type metabolic responses to drugs. *The American Journal of Pathology* 165.3: 901–912.

Thomas, G. N., Ho, S.-Y., Janus, E. D., Lam, K. S., Hedley, A. J., Lam, T. H. 2005. The US national cholesterol education programme adult treatment panel III (NCEP ATP III) prevalence of the metabolic syndrome in a Chinese population. *Diabetes Research and Clinical Practice* 67.3: 251–257.

Thummel, K. E., and Wilkinson, G. R. 1998. *In vitro* and *in vivo* drug interactions involving human CYP3A. *Annual Review of Pharmacology and Toxicology* 38.1: 389–430.

Timbo, B. B., Ross, M. P., McCarthy, P. V., and Lin, C.-T. J. 2006. Dietary supplements in a national survey: prevalence of use and reports of adverse events. *Journal of the American Dietetic Association* 106.12: 1966–1974.

Tolonen, A., Petsalo, A., Turpeinen, M., Uusitalo, J., and Pelkonen, O. 2007. *In vitro* interaction cocktail assay for nine major cytochrome P450 enzymes with 13 probe reactions and a single LC/MSMS run: analytical validation and testing with monoclonal anti-CYP antibodies. *Journal of Mass Spectrometry* 42.7: 960–966.

Tonini, M. 1996. Recent advances in the pharmacology of gastrointestinal prokinetics. *Pharmacological Research* 33.4–5: 217–226.

Totah, R. A., and Rettie, A. E. 2005. Cytochrome P450 2C8: substrates, inhibitors, pharmacogenetics, and clinical relevance. *Clinical Pharmacology and Therapeutics* 77.5: 341–352.

Tripathi, K. D. 2013. Essentials of Medical Pharmacology. JP Medical Ltd. 928-934. Retrieved Nov. 28, 2016, from https://books.google.com.ng/books?id=FfG8AQAAQBAJ&printsec=frontcover&dq=Essentials+of+Medical+Pharmacology.+JP+Medical&hl=en&sa=X&ved=0ah

UKEwjqidPmq8rQAhVSGsAKHc_GAtYQ6AEIGjAA#v=onepage&q=drug%20interactions&f=false

Tsai, P.-J., McIntosh, J., Pearce, P., Camden, B., and Jordan, B. R. 2002. Anthocyanin and antioxidant capacity in Roselle (*Hibiscus sabdariffa* L.) extract. *Food Research International* 35.4: 351–356.

Tseng, T. H., Kao, E. S., Chu, C. Y., Chou, F. P., Wu, H. W. L., and Wang, C. J. 1997. Protective effects of dried flower extracts of Hibiscus sabdariffa L. against oxidative stress in rat primary hepatocytes. *Food and Chemical Toxicology* 35.12: 1159–1164.

Tseng, T. H., Wang, C. J., Kao, E. S., and Chu, H. Y. 1996. Hibiscus protocatechuic acid protects against oxidative damage induced by tert-butylhydroperoxide in rat primary hepatocytes. *Chemico-Biological Interactions* 101.2: 137–148.

Tseng, T.-H., Hsu, J.-D., Lo, M.-H., Chu, C.-Y., Chou, F.-P., Huang, C.-L., and Wang, C. J. 1998. Inhibitory effect of Hibiscus protocatechuic acid on tumor promotion in mouse skin. *Cancer Letters* 126.2: 199–207.

Tsuji, A., and Tamai, I. 1996. Carrier-mediated intestinal transport of drugs. *Pharmaceutical Research* 13.7: 963–977.

Tsutsui, M., Ogawa, S., Inada, Y., Tomioka, E., Kamiyoshi, A., Tanaka, S., Kishida, T., Nishiyama, M., Murakami, M., Kuroda, J., Hashikura, Y., Miyagawa, S., Satoh, F., Shibata, N., and Tagawa, Y. 2006. Characterization of cytochrome P450 expression in murine embryonic stem cell-derived hepatic tissue system. *Drug Metabolism and Disposition* 34.4: 696–701.

Turpault, S., Brian, W., Van Horn, R., Santoni, A., Poitiers, F., Donazzolo, Y. 2009. Pharmacokinetic assessment of a five-probe cocktail for CYPs 1A2, 2C9, 2C19, 2D6 and 3A. *British Journal of Clinical Pharmacology* 68.6: 928–935.

Turpeinen, M., Jouko, U., Jorma, J., and Olavi, P. 2005. Multiple P450 substrates in a single run: rapid and comprehensive in vitro interaction assay. *European Journal of Pharmaceutical Sciences* 24.1: 123–132.

van Ginneken, C. A., and Russel, F. G. 1989. Saturable pharmacokinetics in the renal excretion of drugs. *Clinical Pharmacokinetics* 16.1: 38–54.

von Moltke, L. L., Greenblatt, D. J., Schmider, J., Wright, C. E., Harmatz, J. S., and Shader, R. I. 1998. *In vitro* approaches to predicting drug interactions in vivo. *Biochemical Pharmacology* 55.2: 113–122.

Wallace, A. W., Victory, J. M., and Amsden, G. W. 2003a. Lack of bioequivalence of gatifloxacin when coadministered with calcium-fortified orange juice in healthy volunteers. *The Journal of Clinical Pharmacology* 43.1: 92–96.

Wallace, A. W., Victory, J. M., and Amsden, G. W. 2003b. Lack of bioequivalence when levofloxacin and calcium-fortified orange juice are coadministered to healthy volunteers. *The Journal of Clinical Pharmacology* 43.5: 539–544.

Walsky, R. L., Obach, R. S., Gaman, E. A., Gleeson, J.-P. R., and Proctor, W. R. 2005. Selective inhibition of human cytochrome P4502C8 by montelukast. *Drug Metabolism and Disposition* 33.3: 413–418.

Wang, Z., Gorski, J. C., Hamman, M. A., Huang, S. M., Lesko, L. J., and Hall, S. D. 2001. The effects of St John's wort (*Hypericum perforatum*) on human cytochrome P450 activity. *Clinical Pharmacology and Therapeutics* 70.4: 317–326.

Wen, X., Wang, J.-S., Backman, J. T., Kivistö, K. T., and Neuvonen, P. J. 2001. Gemfibrozil is a potent inhibitor of human cytochrome P450 2C9. *Drug Metabolism and Disposition* 29.11: 1359–1361.

Wienkers, L. C., and Heath, T. G. 2005. Predicting in vivo drug interactions from in vitro drug discovery data. *Nature Reviews Drug Discovery* 4.10: 825–833.

Williams, J. A., Hyland, R., Jones, B. C., Smith, D. A., Hurst, S., Goosen, T. C., Peterkin, V., Koup, J. R., Ball, S. E. 2004. Drug-drug interactions for UDP-glucuronosyltransferase substrates: a pharmacokinetic explanation for typically observed low exposure (AUCi/AUC) ratios. *Drug Metabolism and Disposition* 32.11: 1201–1208.

Wong, R. S., Cheng, G., and Chan, T. Y. 2003. Use of herbal medicines by patients receiving warfarin. *Drug safety* 26.8: 585–588.

Woolfall, R. C. 1964. An approach to product formulation. *Soap Perfum. Cosmet*, 37.10.

Wright, R. A., and Flapan, A. D. 1994. Scandinavian simvastatin study (4S). *Lancet* 344.8939–8940.

Wrighton, S. A., and Stevens, J. C. 1992. The human hepatic cytochromes P450 involved in drug metabolism. *CRC Critical Reviews in Toxicology* 22.1: 1–21.

Wrolstad, R. E., Durst, R. W., and Lee, J. 2005. Tracking color and pigment changes in anthocyanin products. *Trends in Food Science and Technology* 16.9: 423–428.

Wu, X., Cao, G., and Prior, R. L. 2002. Absorption and metabolism of anthocyanins in elderly women after consumption of elderberry or blueberry. *The Journal of Nutrition* 132.7: 1865–1871.

Yan, Z., and Caldwell, G. W. 2001. Metabolism profiling, and cytochrome p450 inhibition and induction in drug discovery. *Current Topics in Medicinal Chemistry* 1.5: 403–425.

Yee, G. C., Stanley, D. L., Pessa, L. J., Costa, T. D., Beltz, S. E., Ruiz, J., and Lowenthal, D. T. 1995. Effect of grapefruit juice on blood cyclosporin concentration. *The Lancet* 345.8955: 955–956.

Yeh, G. Y., Davis, R. B., and Phillips, R. S. 2006. Use of Complementary Therapies in Patients With Cardiovascular Disease. *The American Journal of Cardiology* 98.5: 673–680.

Yin, O. Q. P., Tomlinson, B., Waye, M. M. Y., Chow, A. H. L., and Chow, M. S. S. 2004. Pharmacogenetics and herb-drug interactions: experience with *Ginkgo biloba* and omeprazole. *Pharmacogenetics* 14.12: 841–850.

Yu, K., Chen, F., and Li, C. 2012. Absorption, disposition, and pharmacokinetics of saponins from Chinese medicinal herbs: what do we know and what do we need to know more? *Current Drug Metabolism* 13.5: 577–598.

Zeitlinger, M. A., Derendorf, H., Mouton, J. W., Cars, O., Craig, W. A., Andes, D. 2011. Protein Binding: Do We Ever Learn? *Antimicrobial Agents and Chemotherapy* 55.7: 3067–3074.

Zhang, X. 2000. General guidelines for methodologies on research and evaluation of traditional medicine. *World Health Organization* WHO/EDM/TRM/2000.1. 1–71. Retrieved Aug. 20, 2016, from http://apps.who.int/medicinedocs/pdf/whozip42e/whozip42e.pdf.

Zhang, Z. J., Tan, Q. R., Tong, Y., Wang, X. Y., Wang, H. H., Ho, L. M., Wong, H. K., Feng, Y. B., Wang, D., Ng, R., McAlonan, G. M., Wang, C. Y., and Wong, V. T. 2011. An Epidemiological Study of Concomitant Use of Chinese Medicine and Antipsychotics in Schizophrenic Patients: Implication for Herb-Drug Interaction. *PLoS ONE*, 6.2. Retrieved Aug. 7, 2016, from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3040227/

Zhou, H., Josephy, P. D., Kim, D., and Guengerich, F. P. 2004. Functional characterization of four allelic variants of human cytochrome P450 1A2. *Archives of Biochemistry and Biophysics* 422.1: 23–30.

Zhou, S. F. 2008. Drugs behave as substrates, inhibitors and inducers of human cytochrome P450 3A4. *Current drug metabolism* 9.4: 310–322.

Zhou, S.-F., Yang, L.-P., Wei, M. Q., Duan, W., and Chan, E. 2009. Insights into the structure, function, and regulation of human cytochrome P450 1A2. *Current Drug Metabolism* 10.7: 713–729.

Zhu, B., Ou-Yang, D. S., Chen, X. P., Huang, S. L., Tan, Z. R., He, N., and Zhou H. H. 2001. Assessment of cytochrome P450 activity by a five-drug cocktail approach. *Clinical Pharmacology and Therapeutics* 70.5: 455–461.

.

Appendix A

Sample questionnaire on the survey of the pattern of use of aqueous beverage of *Hibiscus sabdariffa*

DEPARTMENT OF CLINICAL PHARMACY AND PHARMACY ADMINISTRATION
FACULTY OF PHARMACY
UNIVERSITY OF IBADAN

Dear Respondent,

Thank you for taking your time to respond to this survey on the use of ***Zobo* drink** (*aqueous beverage of Hibiscus sabdariffa*). This survey is meant to collect information on what people use *Zobo* drink for, how it is used and what your experiences are on its use. Please be very frank with your answers. Notice that there are no personal identifiers on this questionnaire. The survey should take less than fifteen minutes of your time to complete.

Age (*kindly state it in years*)_____ **Sex:** Male [ ]; Female [ ].

Status: Academic Staff [ ]; Non-Academic staff [ ]; Student [ ].

Level of education completed: Primary [ ]; Secondary [ ]; Tertiary [ ]; Postgraduate [ ].

Faculty: ..... **Department:** .....

Marital status: Not Married [ ]; Married [ ]; Divorced [ ]; Separated [ ]; Widowed [ ]

Religion: Christianity [ ]; Islam [ ]; Traditional worshippers [ ]; Others (specify) .....

Please tick (√) the appropriate answer(s) and where necessary kindly provide detail information to the question asked.		YES	NO
1.	**Have you taken *Zobo* drink before?**		
2.	**If yes, how frequent do you take it?**		
	Everyday		
	Twice a week		
	Once a month		
	Occasionally		
3.	**In what form do you take your *Zobo* drink?**		
	As water beverage (prepared with water)		
	As alcoholic drink (prepared with alcohol)		
4.	**How do you like your *Zobo* drink to be flavoured?**		
	With sugar		
	With garlic		
	With ginger		

	With honey		
	With other types of sweetners (like Canderel, Sweetex e.t.c.)		
5.	**Which of the following is/are the reason(s) why you drink *Zobo*?**		
	Because it contains vitamin C		
	Because it helps in improving fertility		
	Because it helps in lowering blood pressure		
	Because it helps in reducing blood cholesterol		
	Because it helps reducing blood sugar level		
	Because it aids sleep		
	Because it has a relaxing effect on the body		
	Because it helps a pregnant woman to deliver easily		
	Because it helps to relieve menstrual cramps		
	Because it helps to regulate irregular menstration		
	Because it helps to relieve constipation		
	Because it helps in healing pile		
	Because it improves sexual performance		
	Because it can cure liver disease		
	Because it can cure kidney disease		
	Because it can relieve ulcer pain		
	Helps in weight reduction		
	Good for improving eyesight		
	Helps remove bad chemicals from the body (antioxidant effect)		
	It is good as a blood tonic		
	I take it as a beverage		
	Other reasons not mentioned, kindly list them here:		
5.	**Do you sometimes use *Zobo* drink to take medications (drugs)?**		
		YES	NO
6.	**If yes, which of the following drugs have you used *Zobo* drink to take before now?**		
	Antibiotics		
	Pain killer		
	Drugs for hypertension		
	Drugs for diabetes		
	Drugs for heart problem		

	Drugs for stomach ulcer		
	Drug for malaria fever		
	Drugs for asthma		
	Drugs for typhoid fever		
	Drugs for HIV infection		
	Drugs for lowering blood cholesterol		
	Drugs for pile		
	Drugs for tuberculosis		
	Drugs for mental illness		
	Other drugs used with *Zobo* that are not mentioned above, kindly list them here:		
7.	**Have you noticed any discomforting effect after taking *Zobo* before now?**		
8.	**What are the discomforting effects that you have noticed after taking *Zobo* drink?**		
	It causes diarrhoea		
	Vomiting		
	Causes menstrual irregularity		
	Decreases sexual performance		
	Causes sleeplessness		
	Increases menstrual flow		
	Causes tiredness		
	Headache		
	Causes dizziness		
	Causes blurred vision		
	Please list other discomforts experienced after taking *Zobo* that are not listed above, here:		
9.	**Do you take drug every day for a certain illness?**		
10.	**Please mention this illness**		
11.	**Kindly list the drugs that you take for this illness**		
12.	**Has taking *Zobo* drink with other drugs caused you any discomfort?**		
13.	**If yes, kindly mention the kind of discomfort you experienced when you took *Zobo* with**		

	other drugs.		
14.	**Did this discomfort lead to hospitalization?**		
15.	**How long did you stay in the hospital (*Please state it in weeks*)**		
16.	**Do you consider *Zobo* drink as medicinal?**		
17.	**If yes, what do you use *Zobo* drink to cure?**		
18.	**Does it work when used for this purpose?**		

Thank you once again for your time.

Appendix B

Questionnaire to determine the health status of volunteer

1. Would you say your health is Good/Fair /Poor
2. Have you ever been admitted as an in-patient in hospital? Yes/No
3. If yes to question 3, please state the reason

_____

4. Are you on any medication(s) Yes/No
5. If yes to question 4 please list the medication(s)

_____

6. Do you suffer from or have suffered from any of the following
   a. Tuberculosis Yes/No
   b. Schistosomiasis Yes/No
   c. Any respiratory disease Yes/No
   d. Sickle cell disease Yes/No
   e. Allergies Yes/No
   f. Diabetes Yes/No
   g. Any disease of the digestive system Yes/No
   h. Any disease of the heart Yes/No
   i. Any genito-urinary disease Yes/No
   j. Nervous disease Yes/No
7. Is your family a healthy one? Yes/No
8. Has any member of your family suffered from any of the following?
   a. Tuberculosis Yes/No
   b. Diabetes Yes/No
   c. Hypertension Yes/No
   d. Mental illness Yes/No
9. Do you react to any drug(s)? Yes/No
10. List the drugs you react to

_____

11. Have you ever been immunized against any of the following?
   a. Hepatitis Yes/No
   b. Tetanus Yes/No
   c. Yellow fever Yes/No
   d. Cerebrospinal meningitis Yes/No

**Adapted from the Postgraduate School, University of Ibadan Student Entrance Medical Examination*

Appendix C

Informed consent form

IRB Research approval number **UI/EC/11/0073**

This approval will elapse on **11/05/2015**

TITLE OF RESEARCH

***IN VIVO* STUDY OF THE AQUEOUS BEVERAGE OF *Hibiscus sabdariffa* L (family. Malvaceae) USING DRUG COCKTAIL**

NAME OF RESEARCHER:

MR SHOWANDE SEGUN J.
Department of Clinical Pharmacy and Pharmacy Administration.
Faculty of Pharmacy. University of Ibadan. Ibadan

PURPOSE OF RESEARCH

To investigate the Cytochrome P450 isozymes that are inhibited by the administration of aqueous beverage of *Hibiscus sabdariffa* popularly called *Zobo*. To know if drinking *Zobo* will affect the action(s) of other drugs that may be taken with it

PROCEDURE OF RESEARCH

Participants will be divided into two equal groups. The first group will take caffeine and metoprolol or caffeine and simvastatin while the second group will take *Zobo* drink with caffeine and metoprolol or caffeine and simvastatin. Blood samples will be collected from individuals at 4 hours after the administration of caffeine and metoprolol in pre-labeled containers, while for the cocktail of simvastatin and caffeine blood samples will be collected over a 24hr period at 0, 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 24hr. There will be a period of one week break after which the above procedure will be reversed that is, group one will now take *Zobo* and drug cocktail while group two will only take the drug cocktail with water and samples will be collected the same way as above. We hope to recruit **26** participants into the study of caffeine and metoprolol while 6-10 healthy volunteerswill be recruited for the caffeine and simvastatin study..

EXPECTED DURATION OF RESEARCH AND OF PARTICIPANTS INVOLVEMENT

The research on the whole will take about **two** months but your actual involvement in this research will only be for a period of two weeks.

RISK

The drug that you will take in this study have been taken by several volunteers in many other studies and there has not been any report of bad effect, however if you experience any serious side effect and wish to withdraw from the study, kindly alert the principal investigator.

COST TO THE PARTICIPANTS

Your involvement in this study will cost you nothing.

BENEFIT(S)

The goal of this study is to know if taking *Zobo* affects the activities and the way the body handles other drugs. If this is the case then the study result will be published to alert the public. However, we are not sure what we will find.

CONFIDENTIALITY

All information collected in this study will be given code numbers and no name will be recorded. This cannot be linked to you in anyway and your name or any identifier will not be used in any publication or reports from this study.

VOLUNTARINESS

Your participation in this research is entirely voluntary.

DUE INDUCEMENT

You will be compensated for the cost of transportation to and from the study site but you will not be paid any fee for participating in this research.

MODALITY OF PROVIDING TREATMENTS AND ACTION(S) TO BE TAKEN IN CASE OF INJURY OR ADVERSE EVENT(S)

If you suffer any injury as a result of your participation in this research, you will be treated at the University College Hospital (UCH) and the researcher will bear the cost of this treatment.

STATEMENT OF PERSONS OBTAINING INFORMED CONSENT

I have fully explained this research to
_____ and have given sufficient information about risks and benefits, to make an informed decision.
DATE_____ SIGNATURE_____
NAME_____

STATEMENT OF PERSONS GIVING INFORMED CONSENT

I have read the description of the research. I understand that my participation is voluntary. I know enough about the purpose, methods, risks, and benefits of the research to judge that I want to take part in it. I understand that I may freely stop being part of this study at any time. I have received a copy of this consent form.

DATE _____ SIGNATURE _____
NAME _____
WITNESS' SIGNATURE (IF APPLICABLE) _____

WITNESS' NAME (IF APPLICABLE)

_____

DETAILED CONTACT INFORMATION

This research has been approved by the Health Research Ethics Committee at Biode Building, 2nd Floor, Room T10, IMRAT. College of Medicine. University of Ibadan. Email: uiuchirc@yahoo.com ext: 2451.

In addition if you have any question about your participation in this research, you can contact the principal investigator Mr. Showande Segun J. Department of Clinical Pharmacy and Pharmacy Administration. Faculty of Pharmacy. University of Ibadan. Email: pharmseg@yahoo.com phone number 08027887608.

You can also contact the Ag. Head of Department, Dr Titilayo O. Fakeye. Department of Clinical Pharmacy and Pharmacy Administration. Faculty of Pharmacy. University of Ibadan

Appendix D

Percentage enzyme activity remaining – ethanolic extract of *Hibiscus sabdariffa*

Conc [µg/ml]*	OH-MEL**		OH-COU**		OH-BUP**		desEt-AMO**		OH-TOL**		deM-OME**		5OH-OME**		O-deM-DEX**		OH-CLZ**		3OH-C
0	96.8	103.2	100.3	99.7	95.5	104.5	94.2	105.8	101.2	98.8	101.2	98.8	98	102	98.4	101.6	90.9	109.1	99.9
0.001	109.7	97.7	108.6	99.1	109.3	104.2	93.8	90.8	124.6	94.3	111.1	107	108.3	98	117.7	106.8	95.3	108.5	107.3
0.01	103.5	102.2	105.9	104.5	109.8	103.2	94	98.7	110.4	85.3	110.7	100.6	107.1	97.4	99.4	93.4	102.8	102.2	102.4
0.1	116.1	103.9	112.2	109.2	116.9	103.3	90.7	92.2	107.9	99.9	114.7	109.4	116.9	102.7	125.7	102.1	112.9	107.2	115.1
1	92.4	102.5	104	101.7	96.7	102.9	88.8	91.5	94.1	108.3	98.2	102.2	94.5	97.9	95.5	102.3	87.8	94	93
10	91.9	89.2	99.7	98.9	101.6	101.5	89.4	89.6	103	88.1	94.1	99.9	94.7	96.1	90	100.5	87.8	98.4	94.3
100	69.9	84	93.9	94.8	85.5	93.8	85.1	85.7	87	107.1	88.6	95.8	89.4	89.3	81	92	95.9	86.5	88.4
1000	24.9	20.8	66.5	58.3	30.7	24.7	30.1	24.8	48.1	29.9	37.1	33.5	35.8	29.8	30.5	24.9	29.9	28.7	39.3

*Concentration of the dried extract in the incubation

**Percentual enzyme activity remaining, duplicate determinations at each concentration

The data for 7-OH-COU and OH-CLZ is corrected based on the observed suppression of the LC/MS -response at the highest concentrations of the extract

Appendix E

Peak area value for the LC/MS/MS analysis of aqueous extract of *Hibiscus sabdariffa*

	ACET (1A2)	7-OH-COU (2A6)	OH-BUP (2B6)	OH-REPA (2C8)	OH-DICL (2C9)	5-OH-OME (2C19)	DeM-OME (2C19)	O-DeM-DEX (2D6)	3-OH-OME (3A4)	6b-OH-TES (3A4)	1-OH-MDZ (3A4)	SO2-OME (3A4)
solvent	121181	272626	129461	572486	623811	428235	106939	83870	148298	255567	982201	218821
solvent	90759	173077	95424	403883	303510	332856	74146	69334	101982	192480	610143	117089
Hibiscus sabdariffa 1000 µg/mL rep1	27200	153505	49115	111239	370272	243620	66335	41639	87871	146056	769967	121973
Hibiscus sabdariffa 1000 µg/mL rep2	24555	112970	34866	87122	230783	235746	59085	39409	78489	136381	555543	88081
Hibiscus sabdariffa µg/mL rep1	102462	228194	96535	443152	474862	358781	90966	70110	122702	215890	879728	163856
Hibiscus sabdariffa 100 µg/mL rep2	105118	179769	87846	437851	362814	368137	87813	72517	113692	203753	684815	125853
Hibiscus sabdariffa 10 µg/mL rep1	129687	223857	98212	528072	519475	410566	99279	73945	141981	234953	873807	184699
Hibiscus sabdariffa 10 µg/mL rep2	102127	199497	91167	428865	342631	359671	84936	72812	116707	222838	704277	139365
Hibiscus sabdariffa 1 µg/mL rep1	131687	250441	95393	534761	523206	417947	106704	73635	139615	250510	955527	203183
Hibiscus sabdariffa 1 µg/mL rep2	124109	165943	108266	529185	355302	401934	95019	81349	122097	212395	677710	120754
Hibiscus sabdariffa 0.1 µg/mL rep1	140769	250862	110795	542844	547414	438515	112408	80387	149437	241771	945882	208500
Hibiscus sabdariffa 0.1 µg/mL rep2	100391	189334	92588	437420	323599	389036	84633	76169	119717	217516	676532	136663
Hibiscus sabdariffa 0.01 µg/mL rep1	147244	243841	111657	564781	550833	437530	111768	79197	145459	251441	953521	203183
Hibiscus sabdariffa 0.01 µg/mL rep2	121045	156139	105117	498520	309361	391784	93730	79625	116817	203004	567760	113231
Hibiscus sabdariffa 0.001 µg/mL rep1	146845	257946	120555	579317	604143	447968	116464	74792	151481	248715	964584	218571
Hibiscus sabdariffa [HS] 0.001 µg/mL rep2	130414	160122	108810	508500	327811	382673	90714	82173	114315	220494	567275	101075
HS Stock rep 1	120172	188307	81878	447248	425363	385782	98391	75975	131337	238147	752910	164367
HS Stock rep 2	107091	105885	94901	373164	250686	376254	78532	81277	104159	190982	483410	91404

Appendix F

Percentage enzyme activity remaining for CYP isoforms with aqueous extract of *Hibiscus sabdariffa*

	NCE conc (µM)	ACET_(1A2)	7-OH-COU_(2A6)	OH-BUP_(2B6)	OH-REPA_(2C8)	OH-DICL_(2C9)	DeM-OME_(2C19)	5-OH-OME_(2C19)	O-deM-DEX_(2D6)	3-OH-OME_(3A4)	SO2-OME_(3A4)	6b-OH-TES_(3A4)	1-OH-MDZ_(3A4)
solvent	0	114.35%	122.34%	115.14%	117.27%	134.54%	118.11%	112.53%	109.49%	118.51%	130.29%	114.08%	123.37%
solvent	0	85.65%	77.66%	84.86%	82.73%	65.46%	81.89%	87.47%	90.51%	81.49%	69.71%	85.92%	76.63%
Hibiscus sabdariffa [HS] 1000 µg/ml rep1	1000	25.67%	68.88%	43.68%	22.79%	79.86%	73.26%	64.02%	54.36%	70.22%	72.62%	65.20%	96.71%
Hibiscus sabdariffa [HS] 1000 µg/ml rep2	1000	23.17%	50.69%	31.01%	17.85%	49.77%	65.26%	61.95%	51.45%	62.72%	52.44%	60.88%	69.78%
Hibiscus sabdariffa [HS] 100 µg/ml rep1	100	96.69%	102.40%	85.85%	90.78%	102.42%	100.47%	94.28%	91.53%	98.05%	97.56%	96.37%	110.49%
Hibiscus sabdariffa [HS] 100 µg/ml rep2	100	99.20%	80.67%	78.13%	89.69%	78.25%	96.99%	96.74%	94.67%	90.85%	74.93%	90.95%	86.01%
Hibiscus sabdariffa [HS] 10 µg/ml rep1	10	122.38%	100.45%	87.34%	108.17%	112.04%	109.65%	107.89%	96.53%	113.46%	109.97%	104.88%	109.75%
Hibiscus sabdariffa [HS] 10 µg/ml rep2	10	96.37%	89.52%	81.08%	87.85%	73.90%	93.81%	94.51%	95.05%	93.26%	82.98%	99.47%	88.46%
Hibiscus sabdariffa [HS] 1 µg/ml rep1	1	124.27%	112.38%	84.84%	109.54%	112.84%	117.85%	109.83%	96.13%	111.57%	120.97%	111.82%	120.02%
Hibiscus sabdariffa [HS] 1 µg/ml rep2	1	117.12%	74.46%	96.29%	108.40%	76.63%	104.94%	105.62%	106.20%	97.57%	71.90%	94.81%	85.12%
Hibiscus sabdariffa [HS] 0.1 µg/ml rep1	0.1	132.84%	112.57%	98.53%	111.20%	118.06%	124.15%	115.23%	104.94%	119.42%	124.14%	107.92%	118.80%
Hibiscus sabdariffa [HS] 0.1 µg/ml rep2	0.1	94.74%	84.96%	82.34%	89.60%	69.79%	93.47%	102.23%	99.44%	95.67%	81.37%	97.10%	84.97%
Hibiscus sabdariffa [HS] 0.01 µg/ml rep1	0.01	138.95%	109.42%	99.30%	115.69%	118.80%	123.44%	114.97%	103.39%	116.24%	120.97%	112.24%	119.76%
Hibiscus sabdariffa [HS] 0.01 µg/ml rep2	0.01	114.23%	70.06%	93.49%	102.12%	66.72%	103.52%	102.95%	103.95%	93.35%	67.42%	90.62%	71.31%
Hibiscus sabdariffa [HS] 0.001 µg/ml rep1	0.001	138.57%	115.75%	107.21%	118.67%	130.30%	128.63%	117.72%	97.64%	121.05%	130.14%	111.02%	121.15%
Hibiscus sabdariffa [HS] 0.001 µg/ml rep2	0.001	123.07%	71.85%	96.77%	104.16%	70.70%	100.19%	100.56%	107.27%	91.35%	60.18%	98.42%	71.25%
HS Stock rep 1	1000	113.40%	84.50%	72.82%	91.61%	91.74%	108.67%	101.38%	99.18%	104.95%	97.86%	106.30%	94.57%
HS Stock rep 2	1000	101.06%	47.51%	84.40%	76.44%	54.07%	86.74%	98.87%	106.10%	83.23%	54.42%	85.25%	60.72%

Appendix G

Matrix effect of extracts of *Hibiscus sabdariffa* on 7-hydroxy-coumarin

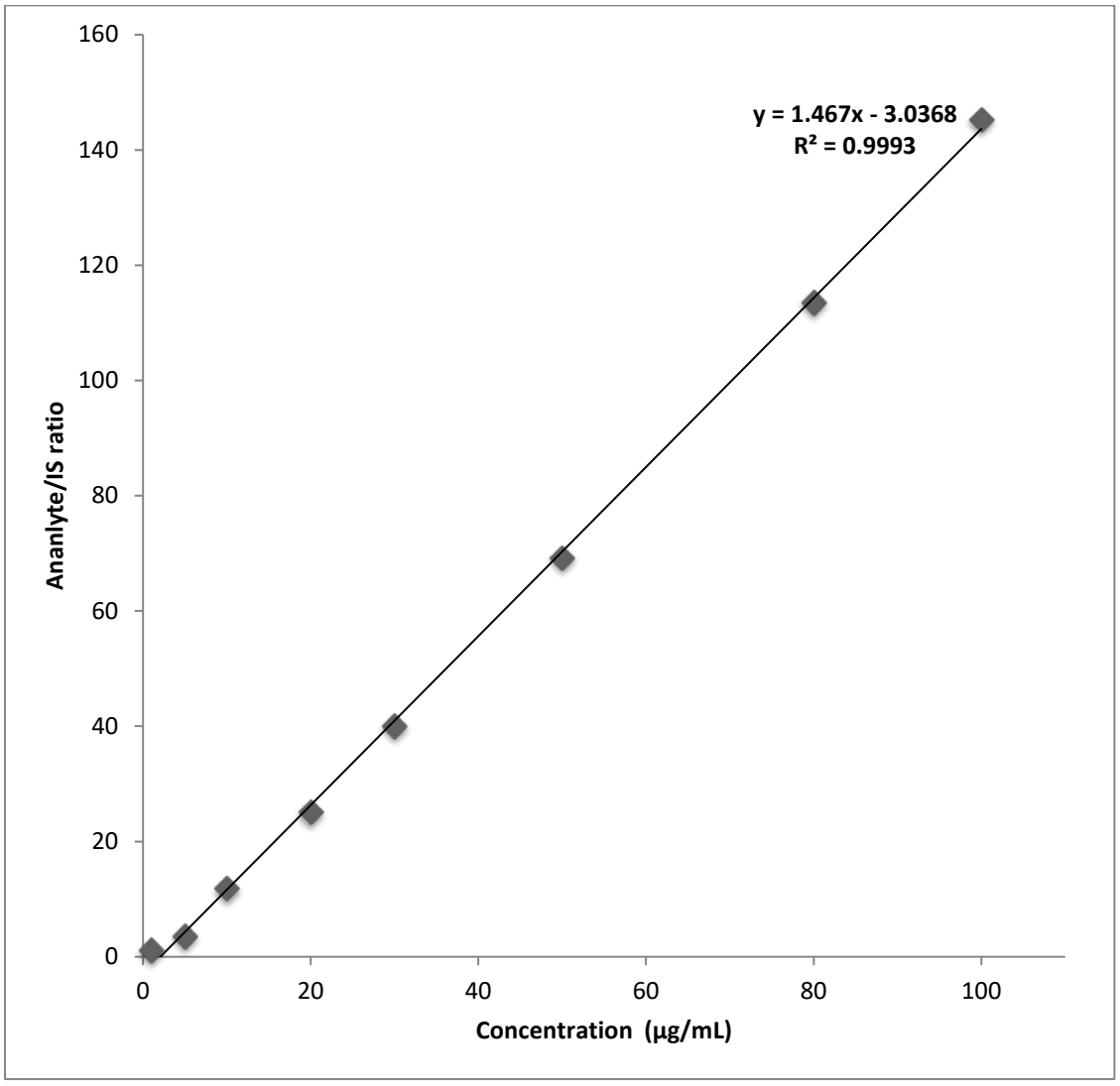
Compound 3: 7-OH-COU (2A6)																	
	#	Name	Sample Text	Quan Trace	RT	Area	IS Area	Response	% of control			Compound 3: 7-OH-COU (2A6)					
1	1	AQU-00951	MeOH K	163.04 > 107.03			26449.086					µg/ml	1	2			
2	2	AQU-00952	MeOH 1	163.04 > 107.03	3.24	14459.272	28740.572	0.5031	100.3			0	100.3	99.7			
3	3	AQU-00953	MeOH 2	163.04 > 107.03	3.24	13949.156	27875.135	0.50042	99.7			0.001	108.6	99.1			
4	4	AQU-00954	100 ng/ml HERB 1	163.04 > 107.03	3.24	14947.961	27442.697	0.5447	108.6			0.01	105.9	104.5			
5	5	AQU-00955	100 ng/ml HERB 2	163.04 > 107.03	3.24	13993.731	28142.053	0.49725	99.1			0.1	112.2	109.2			
6	6	AQU-00956	1 ug/ml HERB 1	163.04 > 107.03	3.24	14476.245	27255.439	0.53113	105.9			1	104.0	101.7			
7	7	AQU-00957	1 ug/ml HERB 2	163.04 > 107.03	3.24	14930.046	28486.596	0.52411	104.5			10	99.7	98.9			
8	8	AQU-00958	10 ug/ml HERB 1	163.04 > 107.03	3.24	15732.583	27940.061	0.56308	112.2			100	87.5	88.4			
9	9	AQU-00959	10 ug/ml HERB 2	163.04 > 107.03	3.23	15246.298	27826.949	0.5479	109.2			1000	49.3	43.2			
10	10	AQU-00960	100 ug/ml HERB 1	163.04 > 107.03	3.24	14537.426	27865.346	0.5217	104.0								
11	11	AQU-00961	100 ug/ml HERB 2	163.04 > 107.03	3.23	13727.042	26888.832	0.51051	101.7			Fixed based on suppression at 100 µg/ml and 1000 µg/ml					
12	12	AQU-00962	1000 ug/ml HERB 1	163.04 > 107.03	3.24	13093.92	26183.332	0.50009	99.7			Compound 3: 7-OH-COU (2A6)					
13	13	AQU-00963	1000 ug/ml HERB 2	163.04 > 107.03	3.24	12714.848	25623.303	0.49622	98.9			µg/ml	1	2			
14	14	AQU-00964	10 mg/ml HERB 1	163.04 > 107.03	3.24	11410.883	25976.732	0.43927	87.5			0	100.3	99.7			
15	15	AQU-00965	10 mg/ml HERB 2	163.04 > 107.03	3.24	11083.376	25001.234	0.44331	88.4			0.001	108.6	99.1			
16	16	AQU-00966	100 mg/ml HERB 1	163.04 > 107.03	3.25	5796.743	23450.021	0.2472	49.3			0.01	105.9	104.5			
17	17	AQU-00967	100 mg/ml HERB 2	163.04 > 107.03	3.23	5190.871	23932.176	0.2169	43.2			0.1	112.2	109.2			
18	18	AQU-00968	100 mg/ml HERB K	163.04 > 107.03	3.24	1849.909	23176.285	0.07982	15.9			1	104.0	101.7			
19	19	AQU-00969	100 ng/ml HERB 1 - spiked after incubation	163.04 > 107.03	3.24	10791.405	24800.816	0.43512	100.0			10	99.7	98.9			
20	20	AQU-00970	1 ug/ml HERB 1 - spiked after incubation	163.04 > 107.03	3.23	10954.68	24162.914	0.45337	104.2			100	93.9	94.8			
21	21	AQU-00971	10 ug/ml HERB 1 - spiked after incubation	163.04 > 107.03	3.24	10746.144	24250.178	0.44314	101.8			1000	66.5	58.3			
22	22	AQU-00972	100 ug/ml HERB 1 - spiked after incubation	163.04 > 107.03	3.23	9555.093	23671.168	0.40366	92.8								
23	23	AQU-00973	1000 ug/ml HERB 1 - spiked after incubation	163.04 > 107.03	3.23	10273.74	23827.443	0.43117	99.1								
24	24	AQU-00974	10 mg/ml HERB 1 - spiked after incubation	163.04 > 107.03	3.24	9214.202	22724.51	0.40547	93.2								
25	25	AQU-00975	100 mg/ml HERB 1 - spiked after incubation	163.04 > 107.03	3.24	6605.025	20485.893	0.32242	74.1	Suppression							

Appendix H

Volunteers hematological and clinical indices for the analysis of caffeine and metoprolol

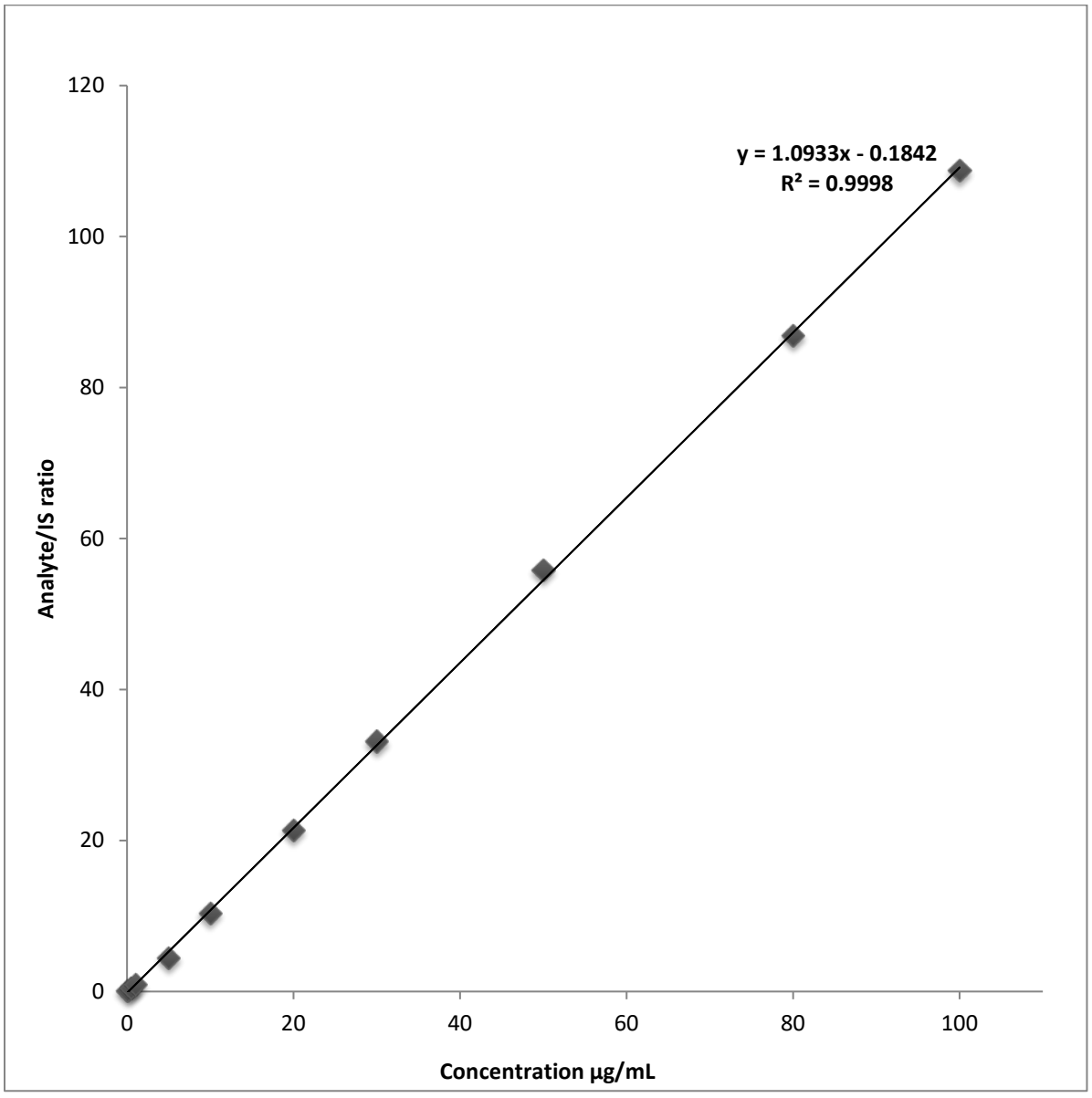
Description	Mean ± SD
Age (yrs)	21.57 ±3.65
Anthropometric and clinical indices	
Body Mass Index (kg/m^2)	22.36 ± 2.34
Waist / Hip ratio	0.83 ± 0.11
Visceral fat (%)	4.25 ± 1.96
Total fat (%)	24.60 ± 9.59
Muscle (%)	35.01 ± 8.10
Fasting Blood Glucose (mg/dL)	72.43 ± 4.28
Average Systolic Blood Pressure (mmHg)	115.03 ± 9.24
Average Diastolic Blood Pressure (mmHg)	66.42 ± 7.71
Pulse (min^{-1})	72.55 ± 10.84
Biochemical indices	
Serum Creatinine (g/dL)	0.86 ± 0.24
Blood Urea Nitrogen (g/dL)	16.19 ± 0.94
Aspartate Transaminase (U/L)	33.43 ± 13.70
Alanine Transaminase (U/L)	25.03 ± 10.35
AST/ALT ratio	1.17 ± 0.48
Hematological indices	
Heamatocrit (%)	41.81 ± 4.18
Hemoglobin (g/dL)	13.83 ± 1.32
Red Blood Cell (/µL)	7.05×10^6 ± 0.68
White Blood Cell (/µL)	4.81×10^3 ± 1.81
Platelets (/µL)	1.31×10^5 ± 0.80
Lymphocytes (%)	69.15 ± 5.15
Neutrophils (%)	26.62 ± 4.78
Monocytes (%)	1.96 ± 0.87
Eosinophil (%)	2.15 ± 1.01

Appendix I



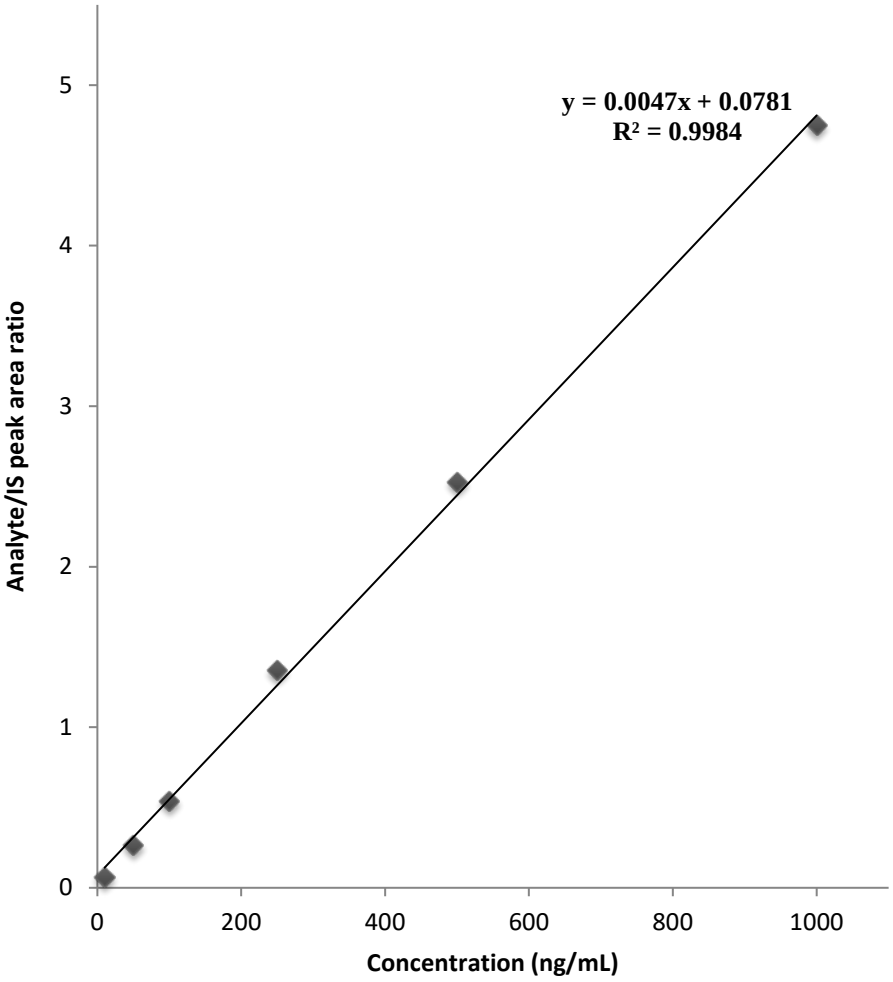
Caffeine calibration curve in spiked plasma used in the analysis of phenotypic metabolic ratio of caffeine

Appendix J



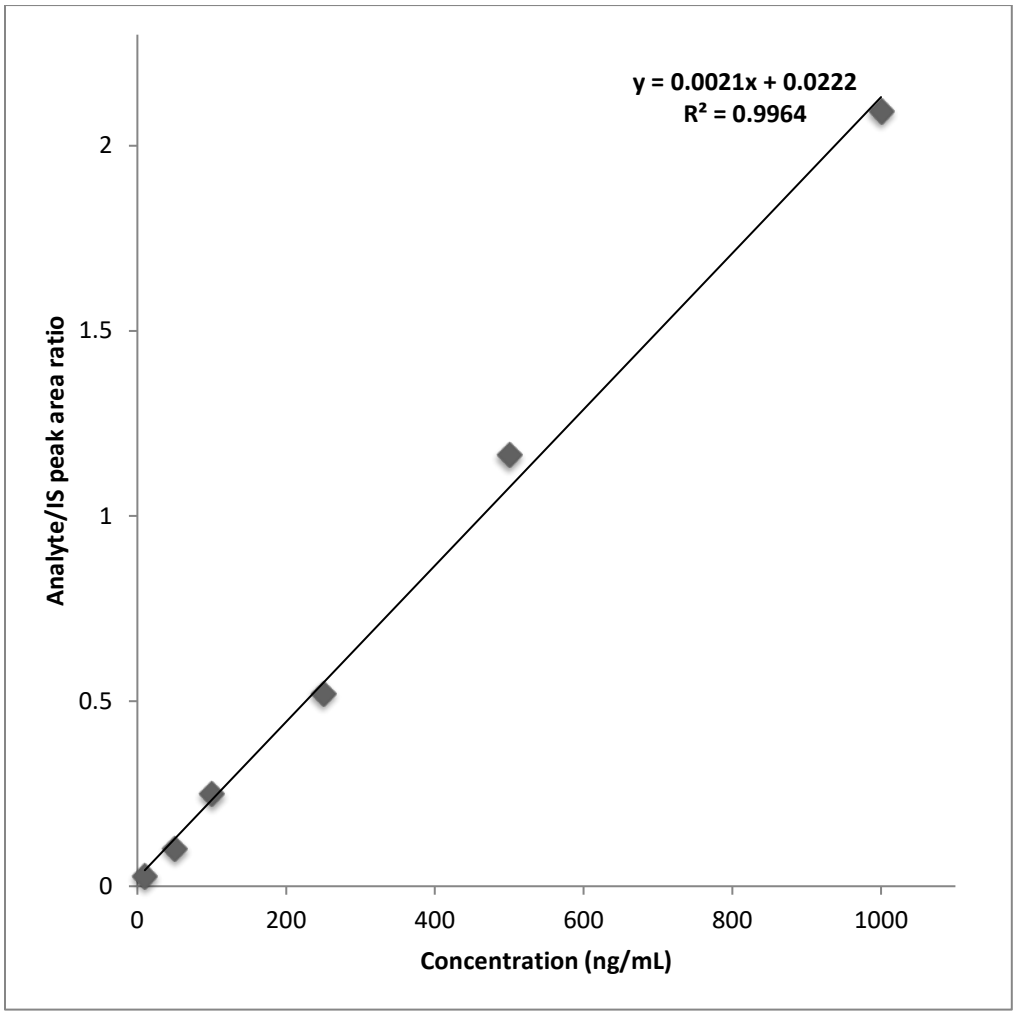
Paraxanthine calibration curve in spiked plasma used in the analysis of phenotypic metabolic ratio of caffeine

Appendix K



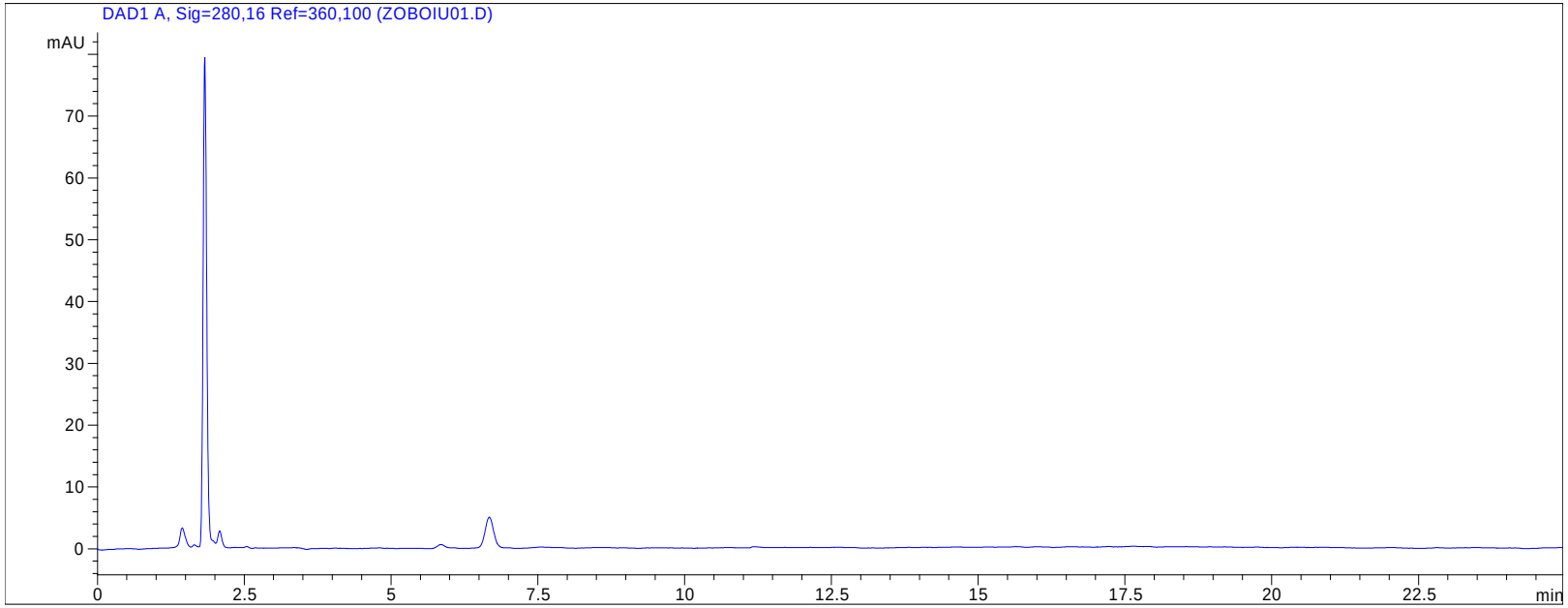
Calibration curve for metoprolol in spiked plasma used in the phenotypic metabolic ratio analysis of metoprolol

Appendix L



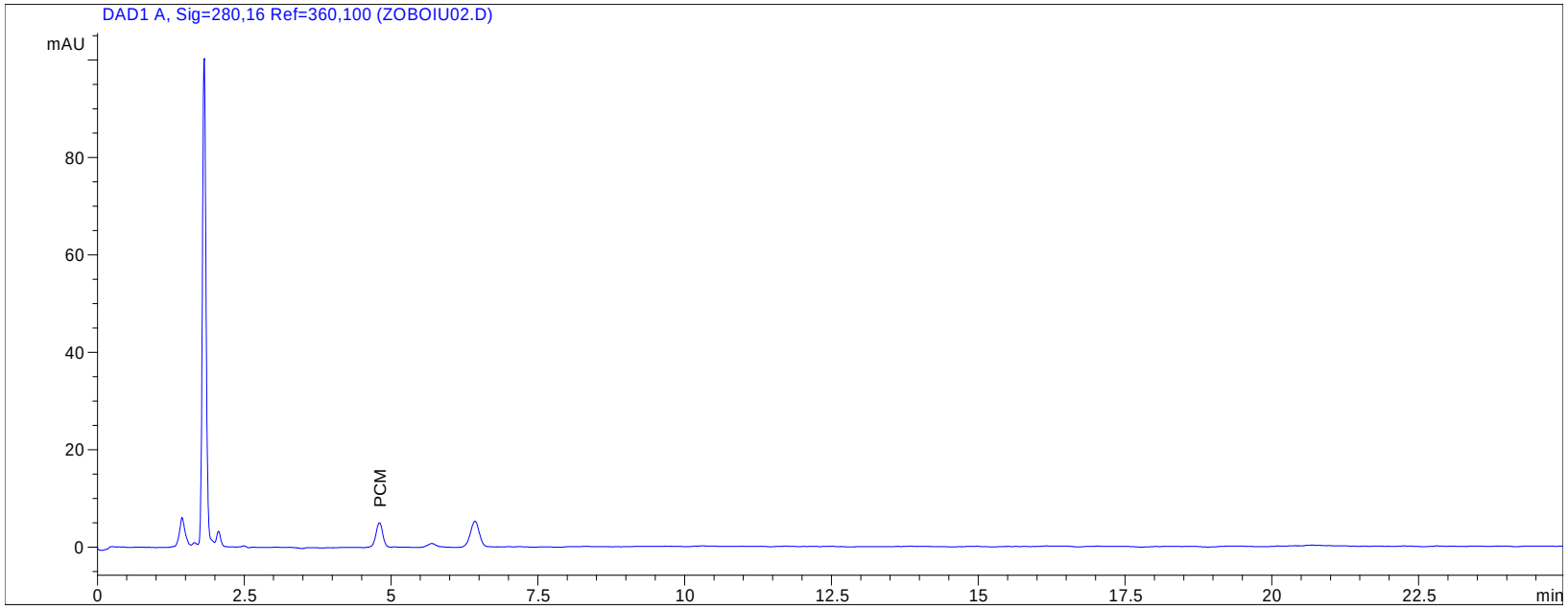
Calibration curve for α-hydroxymetoprolol in spiked plasma used in the phenotypic metabolic ratio analysis of metoprolol

Appendix M



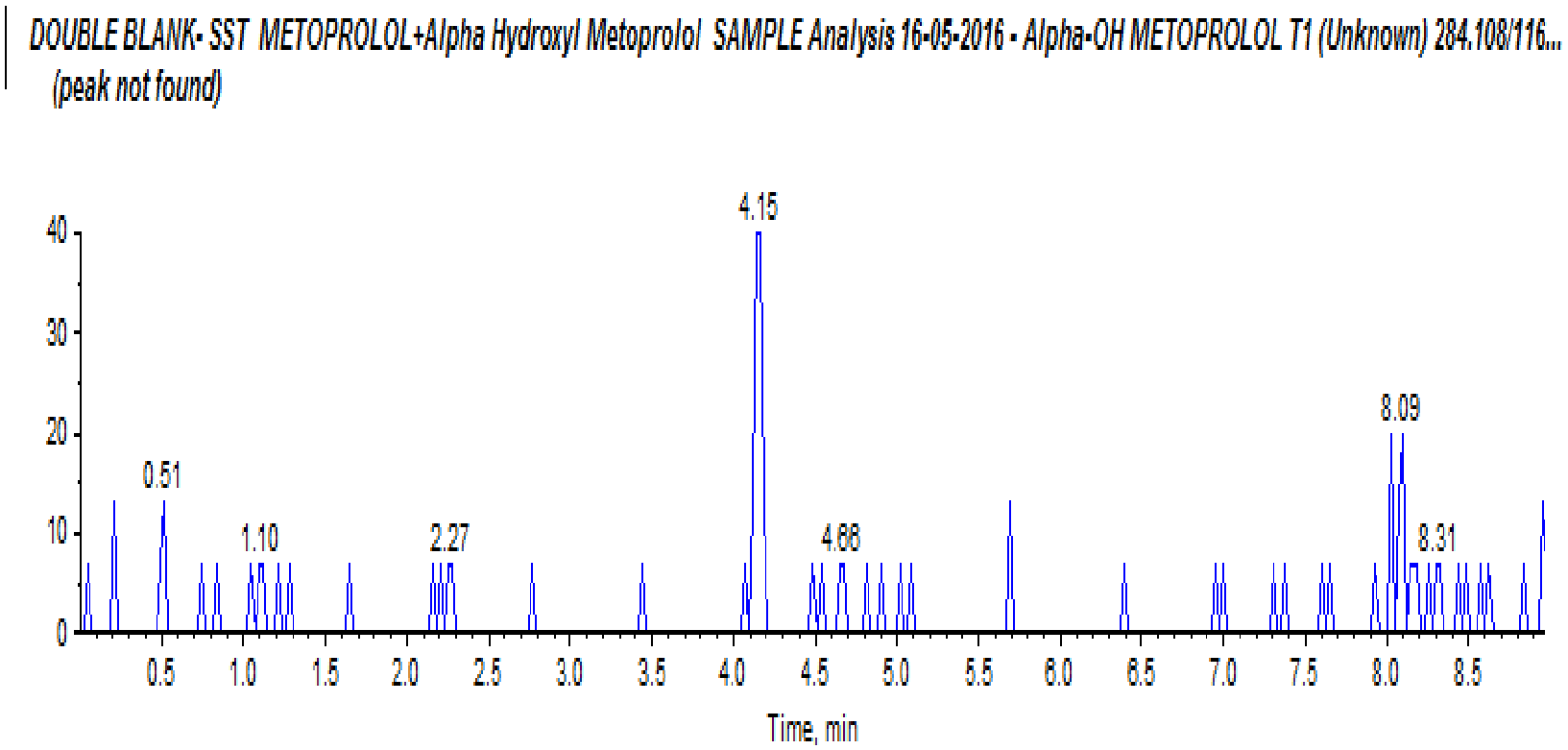
Chromatogram of blank plasma without the spiked internal standard (paracetamol) and the analytes (caffeine and paraxane)

Appendix N



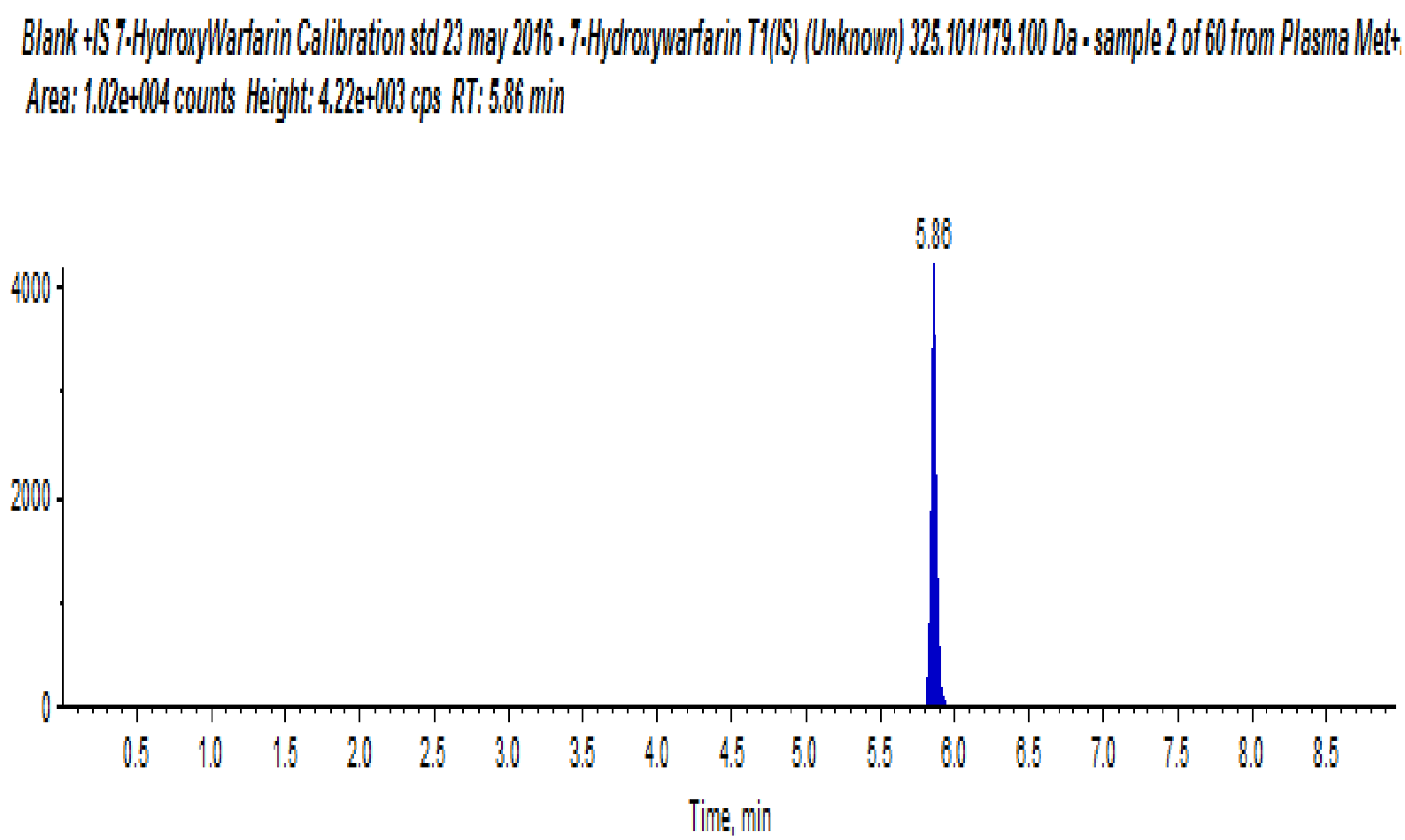
Chromatogram showing plasma spiked with the paracetamol (PCM, internal standard)

Appendix O



LC/MS/MS chromatogram of blank plasma for the analysis of metoprolol

Appendix P



LC/MS/MS chromatogram of blank plasma spiked with 7-hydroxywarfarin (IS) for the analysis of metoprolol.

Appendix Q

Validation parameters of caffeine and paraxanthine for phenotypic metabolic ratio analysis of caffeine

Spiked concentrations	Within-run precision, %CV (n = 6)	Within-run accuracy, %, (n = 6)	Between-run precision, %R.S.D (n = 6)	Between-run accuracy, %, (n = 6)	Recovery, %, (n=6)
Caffeine					
LLOQ (1 µg/mL)	10.94	89.83	1.12	98.33	99.29
LQC (3 µg/mL)	2.31	97.50	1.35	99.33	100.01
MQC (40 µg/mL)	2.61	98.25	2.93	95.89	102.51
HQC (80 µg/mL)	1.94	99.19	0.84	99.60	99.59
Paraxanthine					
LLOQ (0.05 µg/mL)	6.60	93.43	5.12	90.76	89.51
LQC (0.15 µg/mL)	0.58	89.33	3.59	91.60	80.36
MQC (3 µg/mL)	2.94	99.28	4.64	95.78	101.57
HQC (40 µg/mL)	3.28	98.81	4.09	99.19	100.10

Appendix R

Within-run accuracy and precision for metoprolol and α-hydroxymetoprolol for the determination of phenotypic metabolic ratio of metoprolol

Level	Nominal	LQC	Nominal	MQC	Nominal	HQC
Replicate/Units	(ng/mL)	(ng/mL)	(ng/mL)	(ng/mL)	(ng/mL)	(ng/mL)
1	40.00	34.20	400.00	343.00	800.00	739.00
2		36.60		342.00		711.10
3		42.10		417.00		688.00
4		34.10		342.10		684.00
5		34.70		399.90		680.20
6		36.90		393.10		680.00
MEAN		36.43		372.85		697.05
S.D		3.03		34.29		23.59
(% CV)		8.30		9.19		3.38
ACC (%)		91.08		93.21		87.13

ACC - Accuracy

Appendix S

Between-run accuracy and precision for metoprolol and α-hydroxymetoprolol for the determination of phenotypic metabolic ratio of metoprolol

Level	Nominal	LQC	Nominal	MQC	Nominal	HQC
Replicate/Units	(ng/mL)	(ng/mL)	(ng/mL)	(ng/mL)	(ng/mL)	(ng/mL)
1	40.00	43.60	400.00	381.00	800.00	768.01
2		40.00		340.00		707.00
3		41.10		447.00		749.00
4		36.60		371.10		825.00
5		42.30		436.00		680.00
6		44.50		363.00		821.00
MEAN		41.35		389.68		758.33
S.D		2.84		42.50		58.86
(% CV)		6.87		10.91		7.76
ACC (%)		103.38		97.42		94.79

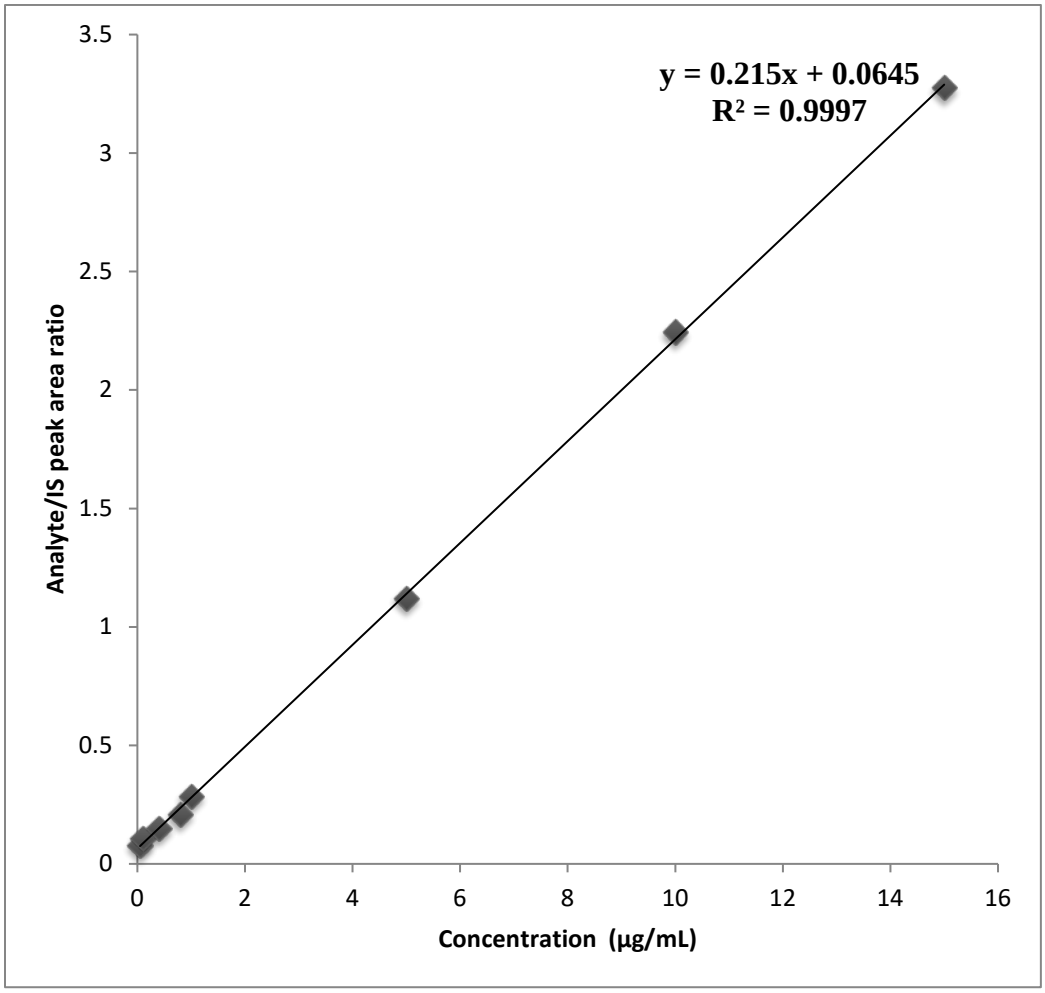
ACC - Accuracy

Appendix T

Volunteers health indices for the pharmacokinetic study of caffeine and simvastatin

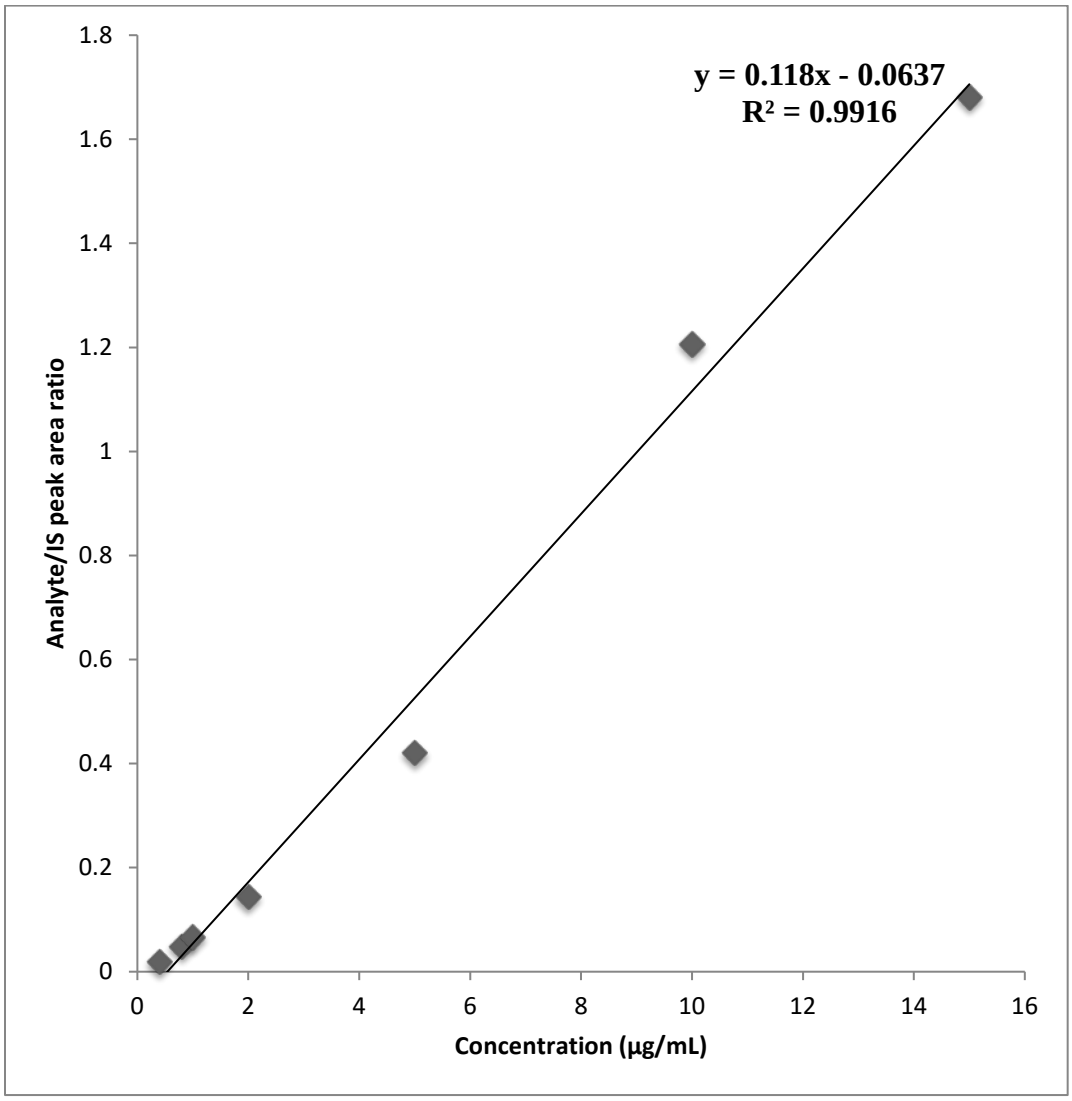
Description	Mean ± SD
Age (yrs)	23.14 ± 3.98
Clinical indices	
Body Mass Index (kg/m^2)	24.07 ± 4.69
Fasting blood glucose (mg/dL)	86.14 ± 12.20
Visceral fat (%)	5.14 ± 2.61
Total fat (%)	21.30 ± 11.45
Muscle (%)	39.36 ± 6.75
Systolic Blood Pressure (mmHg)	125.21 ± 16.73
Diastolic Blood Pressure (mmHg)	71.86 ± 9.36
Pulse (min^{-1})	72.36 ± 10.05
Kidney function indices	
Serum Creatinine (g/dL)	0.67 ± 0.12
Blood Urea Nitrogen (g/dL)	15.17 ± 5.27
Liver function indices	
Aspartate Transaminase (U/L)	9.67 ± 1.75
Alanine Transaminase (U/L)	7.17 ± 2.14
AST/ALT ratio	1.43 ± 0.43
Lipid profile	
Total cholesterol (mg/dL)	115.40 ± 20.32
Triglyceride (mg/dL)	83.71 ± 5.31
High Density Lipoprotein Cholesterol (mg/dL)	49.43 ± 7.30
Low Density Lipoprotein Cholesterol (mg/dL)	55.00 ± 16.09
Very Low Density Lipoprotein Cholesterol (mg/dL)	14.46 ± 6.03
Hematological indices	
Heamatocrit (%)	44.33 ± 2.07
Hemoglobin (g/dL)	14.72 ± 0.48
Red Blood Cell (/µL)	7.58 x 10^6 ± 0.35
White Blood Cell (/µL)	8.63 x 10^3 1.44
Platelets (/µL)	5.40 x 10^5 ± 2.56
Lymphocytes (%)	40.67 ± 12.26
Neutrophils (%)	51.00 ± 9.94
Monocytes (%)	4.67 ± 2.42
Eosinophil (%)	2.33 ± 1.37

Appendix U



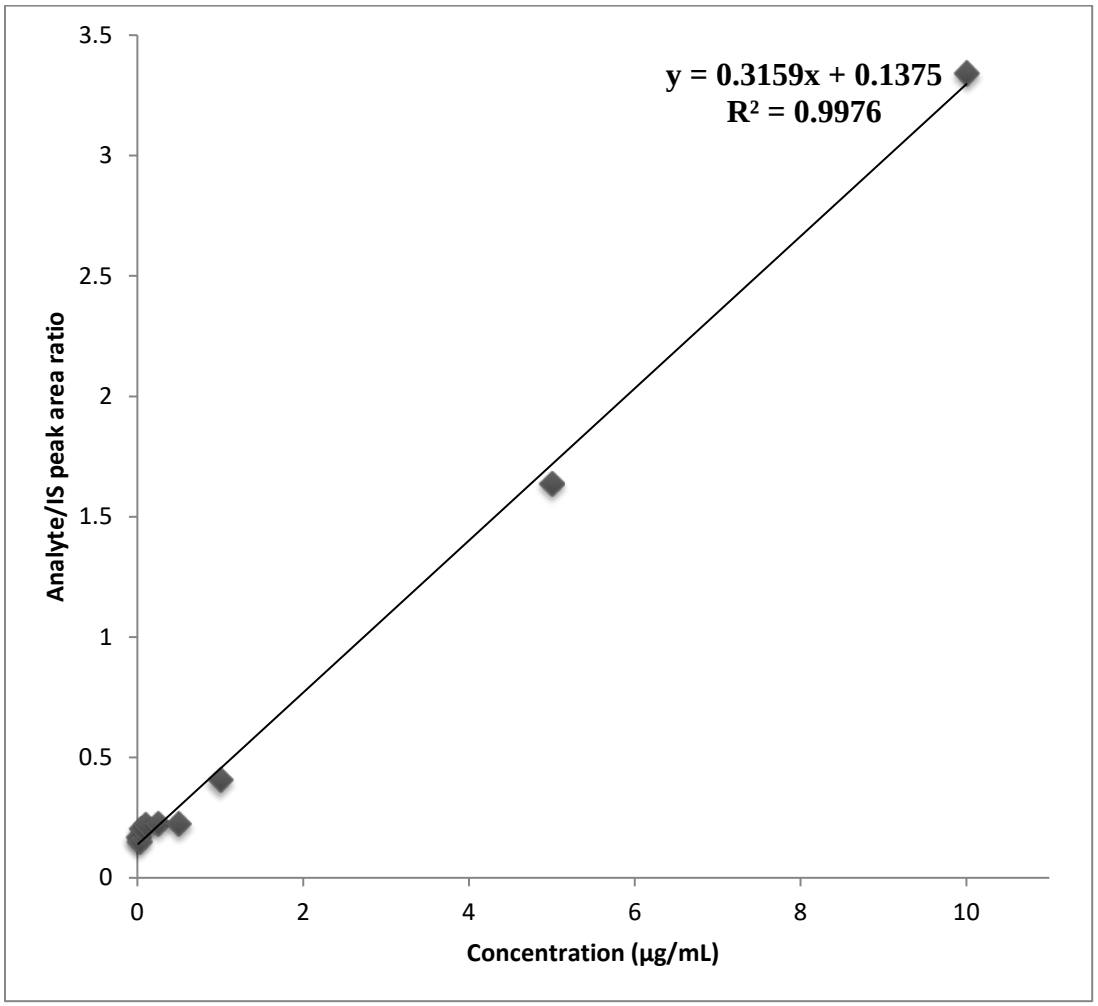
Calibration curve for caffeine in spiked plasma used in pharmacokinetic analysis of caffeine

Appendix V



Calibration curve for paraxanthine in spiked plasma used in pharmacokinetic analysis of caffeine

Appendix W



Calibration curve for simvastatin in spiked plasma used in pharmacokinetic analysis of simvastatin

Appendix X

Validation parameters for caffeine, paraxanthine and simvastatin in the pharmacokinetic analysis of caffeine and simvastatin

Spiked concentrations	Within-run precision, %CV (n = 6)	Within-run accuracy, %, (n = 6)	Between-run precision, %R.S.D (n = 6)	Between-run accuracy, %, (n = 6)	Recovery, %, (n=6)
Caffeine					
LLOQ (0.05 µg/mL)	0.45	100.60	7.14	83.05	97.39
LQC (0.15 µg/mL)	4.84	95.51	6.41	89.26	99.36
MQC (3.50 µg/mL)	3.72	80.21	0.89	98.18	83.38
HQC (10.00 µg/mL)	2.27	86.41	6.69	95.56	84.72
Paraxanthine					
LLOQ (0.40 µg/mL)	4.01	101.32	6.28	96.20	103.21
LQC (1.20 µg/mL)	4.53	101.78	1.32	101.93	101.23
MQC (4.00 µg/mL)	0.33	98.37	3.30	96.97	97.40
HQC (10.00 µg/mL)	0.27	91.07	1.92	82.50	90.79
Simvastatin					
LLOQ (0.01 µg/mL)	11.06	89.76	9.64	91.59	93.89
LQC (0.03 µg/mL)	2.23	103.08	1.37	100.71	69.40
MQC (1.50 µg/mL)	1.20	97.28	2.39	94.18	97.92
HQC (7.00 µg/mL)	7.91	96.37	14.83	90.18	96.60

Appendix Y

Raw lipid profile values for the treatment groups (mg/dL)

Total cholesterol (T_c)				
Treatment Groups	**Day 0** **Mean ± SD**	**Day 30** **Mean ± SD**	**Day 44** **Mean ± SD**	**Day 58** **Mean ± SD**
Zobo 250 mg/kg	95.35 ± 18.22	148.15 ± 20.22	102.34 ± 28.33	114.57 ± 10.19
Zobo 500 mg/kg	80.02 ± 12.58	165.67 ± 22.67	87.30 ± 4.91	104.97 ± 1.65
Simvastatin 10 mg/kg	58.19 ± 8.99	113.53 ± 19.82	87.92 ± 17.17	62.30 ± 16.82
Simvastatin 20 mg/kg	58.49 ± 18.41	99.21 ± 25.99	69.76 ± 14.61	89.33 ± 6.85
Zobo 250 mg/kg + Simvastatin 10 mg/kg	60.54 ± 5.66	143.75 ± 11.59	89.16 ± 21.66	110.21 ± 30.35
Zobo 500 mg/kg + Simvastatin 10 mg/kg	67.50 ± 11.43	157.34 ± 32.36	113.71 ± 8.21	99.90 ± 19.88
Zobo 250 mg/kg + Simvastatin 20 mg/kg	82.19 ± 20.22	170.72 ± 22.95	106.04 ± 7.51	107.05 ± 14.82
Zobo 500 mg/kg + Simvastatin 20 mg/kg	71.72 ± 18.96	112.93 ± 21.47	61.83 ± 12.51	80.92 ± 22.35
Positive Control	73.64 ± 13.06	107.51 ± 8.18	96.15 ± 10.18	90.31 ± 8.58
Negative Control	91.96 ± 19.23	92.57 ± 9.53	81.58 ± 9.69	105.59 ± 12.45

Triglyceride (TG)				
Treatment Groups	**Day 0** **Mean ± SD**	**Day 30** **Mean ± SD**	**Day 44** **Mean ± SD**	**Day 58** **Mean ± SD**
Zobo 250 mg/kg	48.03 ± 13.59	90.73 ± 19.58	53.99 ± 16.88	56.90 ± 29.37
Zobo 500 mg/kg	41.88 ± 16.86	80.42 ± 10.40	48.03 ± 10.44	45.50 ± 23.94
Simvastatin 10 mg/kg	29.81 ± 4.96	74.85 ± 7.56	66.83 ± 36.61	31.04 ± 6.39
Simvastatin 20 mg/kg	22.53 ± 11.42	66.22 ± 12.06	68.81 ± 23.61	40.95 ± 8.61
Zobo 250 mg/kg + Simvastatin 10 mg/kg	39.03 ± 9.38	97.58 ± 11.77	40.40 ± 26.01	71.81 ± 14.26
Zobo 500 mg/kg + Simvastatin 10 mg/kg	33.74 ± 8.10	65.30 ± 15.16	37.22 ± 24.99	77.13 ± 10.24
Zobo 250 mg/kg + Simvastatin 20 mg/kg	50.14 ± 6.66	69.49 ± 17.18	55.32 ± 32.57	71.90 ± 42.42
Zobo 500 mg/kg + Simvastatin 20 mg/kg	33.52 ± 6.04	94.17 ± 16.23	72.22 ± 39.28	83.92 ± 27.90
Positive Control	40.55 ± 2.73	117.69 ± 22.68	93.29 ± 26.66	94.57 ± 15.37
Negative Control	66.40 ± 16.02	60.63 ± 30.85	55.59 ± 10.15	50.93 ± 9.81

High Density Lipoprotein cholesterol (HDL_c)				
Treatment Groups	**Day 0** **Mean ± SD**	**Day 30** **Mean ± SD**	**Day 44** **Mean ± SD**	**Day 58** **Mean ± SD**
Zobo 250 mg/kg	46.14 ± 5.14	22.74 ± 4.66	34.77 ± 8.75	32.89 ± 3.48
Zobo 500 mg/kg	38.21 ± 16.39	20.08 ± 4.11	31.66 ± 2.24	33.67 ± 3.70
Simvastatin 10 mg/kg	20.70 ± 1.31	18.20 ± 1.55	35.53 ± 6.52	21.79 ± 5.16
Simvastatin 20 mg/kg	22.61 ± 6.00	18.30 ± 6.35	30.54 ± 5.54	22.69 ± 4.00
Zobo 250 mg/kg + Simvastatin 10 mg/kg	27.62 ± 7.15	24.48 ± 6.28	36.78 ± 6.21	25.08 ± 4.36
Zobo 500 mg/kg + Simvastatin 10 mg/kg	21.98 ± 4.73	17.86 ± 2.73	32.32 ± 2.10	25.18 ± 4.43
Zobo 250 mg/kg + Simvastatin 20 mg/kg	29.67 ± 7.44	25.09 ± 6.68	38.38 ± 2.26	29.21 ± 1.60
Zobo 500 mg/kg + Simvastatin 20 mg/kg	26.00 ± 10.25	23.95 ± 7.02	34.62 ± 3.38	27.08 ± 3.37
Positive Control	32.84 ± 2.67	28.31 ± 3.67	28.82 ± 2.03	27.89 ± 2.00
Negative Control	39.35 ± 13.62	30.29 ± 6.43	40.46 ± 8.76	37.51 ± 6.48

Low Density Lipoprotein cholesterol (LDL_c)				
Treatment Groups	**Day 0** **Mean ± SD**	**Day 30** **Mean ± SD**	**Day 44** **Mean ± SD**	**Day 58** **Mean ± SD**
Zobo 250 mg/kg	39.60 ± 19.84	107.27 ± 13.89	56.77 ± 20.35	70.30 ± 8.07
Zobo 500 mg/kg	33.43 ± 17.52	129.51 ± 20.31	46.05 ± 4.29	62.21 ± 2.59
Simvastatin 10 mg/kg	31.54 ± 10.09	80.37 ± 19.12	39.03 ± 11.57	34.31 ± 11.78
Simvastatin 20 mg/kg	31.38 ± 10.58	67.67 ± 21.46	25.46 ± 14.56	58.45 ± 8.40
Zobo 250 mg/kg + Simvastatin 10 mg/kg	25.12 ± 9.47	99.75 ± 8.44	44.30 ± 18.95	70.77 ± 28.90
Zobo 500 mg/kg + Simvastatin 10 mg/kg	38.78 ± 8.69	126.42 ± 36.63	73.95 ± 9.11	59.29 ± 21.70
Zobo 250 mg/kg + Simvastatin 20 mg/kg	42.50 ± 15.78	131.74 ± 17.70	56.61 ± 10.39	63.47 ± 15.51
Zobo 500 mg/kg + Simvastatin 20 mg/kg	39.02 ± 19.15	70.15 ± 16.13	12.77 ± 3.85	37.06 ± 13.87
Positive Control	32.69 ± 12.65	55.66 ± 10.79	48.68 ± 11.64	43.51 ± 4.53
Negative Control	39.33 ± 12.54	50.16 ± 13.65	30.01 ± 8.64	57.90 ± 9.49

Appendix Z

Quantitation of caffeine and metoprolol in tablet dosage form using UV – Spectroscopy

PARAMETER	RESULTS	
	CAFFEINE	METOPROLOL
Beer's Limit	0 – 30 µg/ml	0 – 20 µg/ml
Λ-max	273nm	274nm
Correlation Coefficient	0.9956	0.9991
Regression Equation	y = 0.05707x - 0.01321	y = 0.005809x + 0.002120
Slope	0.05707	0.005809
Intercept	-0.01321	0.00212
L.O.D	0.03007	0.86334
L.O.Q	0.09112	2.1618
Precision (%RSD)	1.62	7.407
Intra-day	1.795	
Inter-day	1.7275	
Robustness (%RSD)	1.5	6.893
Concentration of sample	13.273 µg/ml	7.5757 µg/ml
Mass in tablet	176.973 mg	37.800 mg
% Deviation from proposed	-11.513	-24.4