EFFECTS OF *MITRAGYNA SPECIOSA* EXTRACTS ON DRUG METABOLIZING ENZYMES

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EFFECTS OF *MITRAGYNA SPECIOSA* EXTRACTS ON DRUG METABOLIZING ENZYMES

by

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LIST OF SYMBOLS AND ABBREVIATIONS

%	: Percentage sign
ºC	: Degree celcius
µg	: Microgram
µg/mL	: Microgram per milliliter
µ	: Microliter
Ad libitum	: To be taken as wanted
$AlCl_3$	: Aluminium chloride
ANOVA	: One way analysis of variance
APND	: Aminopyrine N-demethylase
ATP	: Adenosine triphosphate
BSA	: Bovne serum albumin
cm	: Centimeter
cm^{-1}	: Recripocal centimeter (units of wavenumber)
cDNA	: Complementary deoxyribonucleic acid
CDNB	: 1-Chloro-2,4-dinitrobenzene
CNS	: Central nervous system
CO	: Carbon monoxide
$CuSO_4.5H_2O$	: Copper(II)sulfate pentahydrate
CYP450	: Cytochrome P450
DCA	: Drug Control Authority
DPPH	: 2,2-diphenyl-1-picryl-hydrazyl
DNA	: Deoxyribonucleic acid
g	: Gravity
GC-MS	: Gas chromatography-mass spectroscopy
GIT	: Gastrointestinal tract
GSH	: Glutathione
GST	: Glutathione S-transferase
g	: Grams
h	: Hour(s)
HIV	: Human immunodeficiency virus
KCl	: Potassium chloride
IC_{50}	: Concentration required to inhibit 50 % radical scavenging effect or enzyme activity
m	: Meter
M. speciosa	: *Mitragyna speciosa*
min	: Minutes
Mg	: Magnesium
mg CE/g	: Milligram catechin equivalents in 1 gram of sample
$MgCl_2$	: Magnesium chloride
mg GAE/g	: Milligram gallic acid equivalents in 1 gram of sample
mg/kg	: Dose (weight of test substance in milligrams per unit weight of test animal)
mg/mL	: Concentration (weight of test substance in milligrams per volume of test concentration)

mL	: Milliliter
mL/kg	: Milliliter per kilogram
mL/min	: Milliliter per minute
mm	: Millimeter
mM	: Millimolar
NaCl	: Sodium chloride
Na_2CO_3	: Sodium carbonate
NCI	: National Cancer Institute
NADPH	: Nicotinamide adenine dinucleotide phosphate (reduced)
NADPHRS	: NADPH regeneration system
$NADP^+$	: Nicotinamide adenine dinucleotide phosphate
NaK Tartrate	: Sodium potassium tartrate
NaOH	: Sodium hydroxide
$NaNO_3$	: Sodium nitrate
ND	: Not determined
nmol	: Nanomole
nm	: Nanometer
No.	: Number
NSAID	: Non-steroidal anti-inflammatory drug
p.o.	: Oral administration
*p*NP	: *Para*-nitrophenol
ppm	: Part per million
ROS	: Reactive oxygen species
s	: Seconds
SD	: Standard deviation
SSRI	: Selective serotonin reuptake inhibitor
spp.	: Species
TCM	: Traditional Chinese medicines
U/mL	: Enzyme unit per milliliter
UDPGA	: UDP-glucuronic acid
UGT	: UDP-glucuronosyl transferase
US FDA	: United States Food and Drug Administration
UV	: Ultraviolet
vs.	: Versus
v/v	: Volume per volume
WHO	: World Health Organization
w/v	: Weight per volume

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LIST OF PUBLICATIONS

Journals

1. Parthasarathy, S., Bin Azizi, J., Ramanathan, S., Ismail, S., Sasidharan, S., Said, M. I., & Mansor, S. M. (2009). Evaluation of antioxidant and antibacterial activities of aqueous, methanolic and alkaloid extracts from *Mitragyna speciosa* (Rubiaceae family) leaves. *Molecules, 14*, 3964-3974.

2. Parthasarathy, S., Ramanathan, S., Chandran, C. S., Azizi, J., Mordi, M. N., Ismail, S., Sasidharan, S., Ikram, M. S., & Mansor, S. M. (2009). Evaluation of antioxidant activity and total phenolic capacity of ketum leaf extracts. *Malaysian Journal of Pharmacy, 11*, S5-S6.

3. Azizi, J., Ismail, S., Mordi, M. N., Ramanathan, S., Said, M. I. M., & Mansor, S. M. (2010). *In vitro* and *in vivo* effects of three different *Mitragyna speciosa* Korth leaf extracts on phase II drug metabolizing enzymes-glutathione transferases (GSTs). *Molecules, 15*, 432-441.

4. Hanapi, N. A., Azizi, J., Ismail, S., & Mansor, S. M. (2010). Evaluation of selected Malaysian medicinal plants on phase I drug metabolizing enzymes, CYP2C9, CYP2D6 and CYP3A4 activities *in vitro*. *International Journal of Pharmacology, 6*, 490-495.

Conferences

1. Azizi, J., Ismail, S., & Mansor, S. M. (2008). *The effect of Mitragyna speciosa methanolic extract on glutathione-s-transferase in vitro.* Paper presented at the 2nd USM Penang International Postgraduate Convention, Universiti Sains Malaysia.

2. Hanapi, N. A., Azizi, J., Ismail, S., & Mansor, S. M. (2009). *In vitro effect of Mitragyna speciosa methanolic, aqueous and total alkaloid extract on CYP3A4.* Paper presented at the 4th Global Summit on Medicinal and Aromatic Plants, Kuching, Sarawak.

3. Ismail, S., Hanapi, N. A., Azizi, J., Mansor, S. M., & Mahmud, R. (2010). *Inhibition of Human Cytochrome P450 Isoforms by Mitragyna speciosa Extracts.* Paper presented at the 9th International ISSX Meeting, Istanbul, Turkey.

4. Azizi, J., Ismail, S., Mordi, M. N., Ramanathan, S., Said, M. I. M., & Mansor, S. M. (2010). *Inhibition of three main CYP450 isoforms by methanolic, aqueous and total alkaloid extracts of Mitragyna speciosa Korth.* Paper presented at the 24th Scientific Meeting of the Malaysian Society of Pharmacology & Physiology, SACC Convention Centre, Shah Alam.

5. Azizi, J., Ismail, S., Mordi, M. N., Ramanathan, S., & Mansor, S. M. (2011). *Mitragyna speciosa methanolic, aqueous and total alkaloid extracts induced the specific activity of UDP-glucuronyl transferase in male Sprague Dawley rats.* Paper presented at the 25th Scientific Meeting of the Malaysian Society of Pharmacology & Physiology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia.

KESAN EKSTRAK *MITRAGYNA SPECIOSA* KE ATAS ENZIM METABOLISME DRUG

ABSTRAK

Daun *M. speciosa* (ketum) telah digunakan secara tradisional di Malaysia dan Thailand untuk meredakan ketagihan candu dan sebagai pengganti candu di saat ketiadaan candu. Daun *M. speciosa* telah disalahgunakan oleh penagih dadah kerana terdapatnya beberapa alkaloid (terutamanya mitraginina) dari tumbuhan ini yang mempunyai kesan seakan candu dan kokain. Hal ini membawa kepada larangan penggunaan daun *M. speciosa* di Malaysia sejak tahun 2004 kerana pengambilan *M. speciosa* telah dianggap membawa kepada penyalahgunaan dadah lain seperti ganja dan heroin. Dalam kajian ini, kesan *in vitro* dan *in vivo* ekstrak metanol, akues dan alkaloid total *M speciosa* ke atas enzim metabolisme drug, iaitu sitokrom P450 (CYP450), UDP-glukuronosil transferase (UGT) dan glutation S-transferase (GST) telah dinilai di dalam fraksi sitosol dan mikrosom hati tikus. Aminopirina, *p*-nitrofenol (*p*NP) dan 1-kloro-2,4-dinitrobenzena (CDNB) masing-masing digunakan sebagai substrat prob dalam esei enzim aminopirina N-demetilase (APND), UDP-glukuronosil transferase (UGT) dan glutation S-transferase (GST). Tambahan pula, terbitan pelbagai lusiferina digunakan sebagai substrat prob untuk menguji secara *in vitro* lima isofom utama CYP450 manusia di dalam sistem bakulosom. Selanjutnya, mitraginina juga diuji secara *in vitro* untuk melihat kemungkinan ia menghalang aktiviti APND, isofom CYP450 manusia, UGT dan GST. Oleh kerana berbagai penerbitan telah menunjukkan kemampuan tumbuh-tumbuhan ubat untuk memodulasi aktiviti enzim metabolisme drug adalah kerana kandungan antioksidannya, maka kandungan fenol total, kandungan flavonoid total dan kapasiti memusnahkan DPPH untuk ketiga-tiga ekstrak *M. speciosa* turut dinilai. Penilaian

aktiviti enzim dan kajian antioksidan dijalankan menggunakan kaedah penyerapan dan luminesen. Daripada ketiga-tiga ekstrak *M. speciosa*, ekstrak metanol adalah yang paling berkesan dalam merencat aktiviti APND, UGT dan GST secara *in vitro* diikuti oleh ekstrak akues dan alkaloid total. Walaubagaimanapun, nilai IC50 hanya boleh diperolehi untuk ekstrak metanol sahaja di dalam kajian perencatan APND (595.30±30.78 µg/mL) dan tidak di dalam kajian yang lain. Ini adalah disebabkan oleh peratusan perencatan enzim kurang dari 70%. Selanjutnya, ekstrak metanol *M. speciosa* juga menunjukkan kandungan flavonoid total tertinggi (90.29±2.34 mg CE/g ekstrak) dan nilai IC_{50} terendah untuk kapasiti memusnahkan DPPH (24.89±1.57 mg/mL). Mitraginina sebaliknya menunjukkan kurang perencatan pada aktiviti APND dan UGT tetapi tidak merencat aktiviti GST. Tambahan lagi, isofom CYP2D6 adalah yang paling direncat oleh ekstrak metanol, akues dan alkaloid total *M. speciosa*, dan mitraginina dengan nilai IC_{50} masing-masing 0.72±0.10, 29.72±4.02, 0.21±0.03 dan 5.99±1.02 (15.02±2.56 µM) mg/mL. Berbeza dengan kajian secara *in vitro*, rawatan oral ke atas tikus jantan Sprague Dawley selama 14 hari dengan 50, 100 dan 200 mg/kg ekstrak metanol dan akues, dan dengan 5, 10 dan 20 mg /kg ekstrak alkaloid total menunjukkan peningkatan ke atas aktiviti APND dan aktiviti UGT tetapi tidak pada aktiviti GST. Daripada hasil kajian ini, boleh disimpulkan bahawa mekanisme modulasi yang berbeza ke atas enzim metabolisme drug mungkin terlibat yang menyebabkan perbezaan keputusan di dalam kajian *in vitro* dan *in vivo*.

EFFECTS OF *MITRAGYNA SPECIOSA* EXTRACTS ON DRUG METABOLIZING ENZYMES

ABSTRACT

M. speciosa (ketum) leaves have been employed traditionally in Malaysia and Thailand to wean opium addiction and as a substitute for opium when opium is unavailable. *M. speciosa* leaves have been abused by drug addicts since some of the alkaloids (mainly mitragynine) from the plant possess opiate and cocaine like effects. These bring to its prohibition in Malaysia in 2004 since consumption of *M. speciosa* leaves has been perceived to lead to the abuse of other drugs such as cannabis and heroin. In the current study, the *in vitro* and *in vivo* effects of *M. speciosa* methanolic, aqueous and total alkaloid extracts on drug metabolizing enzymes, namely cytochrome P450 (CYP450), UDP-glucuronosyl transferase (UGT) and glutathione S-transferase (GST) had been evaluated in rat liver cytosolic fraction and microsomes. Aminopyrine, *p*-nitrophenol (*p*NP) and 1-chloro-2,4-dinitrobenzene (CDNB) were employed as probe substrates in aminopyrine N-demethylase (APND), UDP-glucuronosyl transferase (UGT) and glutathione S-transferase (GST) enzyme assays respectively. In addition, various luciferin derivatives were employed as probe substrates to assay five main human CYP450 isoforms in baculosomes systems *in vitro*. Furthermore, mitragynine was also tested *in vitro* for its likelihood to inhibit APND, human CYP450 isoforms, UGT and GST activity. The total phenolics content, total flavonoids content and DPPH scavenging capacity for the three *M. speciosa* extracts were also evaluated. This is because various publications have implicated the antioxidant content of medicinal herbs to be responsible for the modulation of drug metabolizing enzymes activites. The assessment of the enzyme activity and antioxidant study were conducted using

absorbance and luminescent methods. Out of the three *M. speciosa* extracts, the methanolic extract is the most effective in inhibiting the APND, UGT and GST activity *in vitro* followed by aqueous and total alkaloid extracts. However, IC_{50} value could only be derived for methanolic extract in APND study (595.30±30.78 µg/mL) and not in other studies. This is due to the enzyme percentage inhibitions were less than 70%. In addition, *M. speciosa* methanolic extract exhibited the highest total phenolic content (97.48±2.86 mg GAE/g extract), the highest total flavonoids content (90.29±2.34 mg CE/g extract) and the lowest IC_{50} value for the DPPH antioxidant scavenging capacity (24.89±1.57 µg/mL). Mitragynine on the other hand, showed low inhibition on APND and UGT activities but had no inhibition on GST activity. Additionally, CYP2D6 isoform is the most inhibited by *M. speciosa* methanolic, aqueous and total alkaloid extracts, and mitragynine with respective IC_{50} value of 0.72±0.10, 29.72±4.02, 0.21±0.03 and 5.99±1.02 (15.02±2.56 µM). In contrast to the *in vitro* study, oral treatment of male Sprague Dawley rats for 14 days with 50, 100 and 200 mg/kg of methanolic and aqueous extracts, and with 5, 10 and 20 mg/kg of total alkaloid extract showed increment of APND and UGT activities, but not GST activity. It can be concluded that different mechanisms of modulation of drug metabolizing enzymes might have taken place leading to the disparity in the results between *in vitro* and *in vivo* study.

CHAPTER ONE

INTRODUCTION

Many of the clinically important drug-drug interactions result from perturbations of drug metabolism, involving either induction or inhibition of drug metabolizing enzymes, principally the cytochrome P450s (Woolf, 1999). The usage of herbal medicines as an alternative way to treat multiple ailments and diseases has been a phenomenon worldwide; Malaysia is not exceptional (Aziz & Tey, 2009). Herbal medicines are deemed to be safer for human than conventional medicines owing to public belief that herbal medicines are not synthetic chemicals; since it is more natural (Lynch & Berry, 2007). However, this is not the case, in view of the fact that herbs contains various type of chemicals, although natural, have potentials to interfere with pharmacokinetic properties of other drugs (Venkataramanan, et al., 2006). The pharmacokinetics interactions of herbal medicines with other medicines could occur when there are alterations in absorption, metabolism, distribution and excretion of drugs (Zhou, Koh, Gao, Gong, & Lee, 2004). Alteration of drug metabolism by interference with herbal medicines is significantly important. This is because, most of the reported clinical adverse drug interactions were due to the induction or inhibition of drug metabolizing enzymes, specifically cytochrome P450s (CYP450), UDP-glucuronosyl transferase (UGT) and glutathione S-transfrease (GST) (Woolf, 1999). *Mitragyna speciosa* has been employed as an herbal medicine by natives in Southeast Asia particularly in Malaysia and Thailand to wean opium addiction and as a substitute for opium when opium is unavailable (Reanmongkol, Keawpradub, & Sawangjaroen, 2007; Suwanlert, 1975; Tsuchiya, et al., 2002). Pharmacological effects of *M. speciosa* extract are largely due to

the main principle alkaloid, mitragynine which acts mainly on mu and delta opioid receptors in *in vivo* and *in vitro* studies and possess analgesic effect (Takayama, et al., 2002; Yamamoto, et al., 1999). Most of the studies on *M. speciosa* were focused on the chemical and medicinal aspects and there is no study done on the effects of of this plant extracts and its principle alkaloid on drug metabolizing enzymes. Knowledge of the modulation of drug metabolizing enzymes by *M. speciosa* extracts and mitragynine is a fundamental requirement to the success of potential drug candidates (Plant, 2004), as poorly understood safety features could lead to unpredicted failures in clinical trial. With this in view, it is essential to have some sort of procedures to screen *M. speciosa* extracts and mitragynine against drug metabolizing enzymes, thus, avoiding the serious and significant clinical herb-drug interactions. Herein, in this study, *M. speciosa* methanolic, aqueous and total alkaloid extracts and its principle alkaloid, mitragynine were investigated for their effects on aminopyrine N-demethylation, UDP-glucuronic acid conjugation and glutathione conjugation *in vitro*. Additionally, further efforts were taken by using five main human CYP450 isoforms, in order to see the potential inhibition of *M. speciosa* extracts and mitragynine on those isoforms *in vitro*. Besides *in vitro* study, *in vivo* study was also conducted by treating the male Sprague-Dawley rats for 14 days with *M. speciosa* methanolic, aqueous and total alkaloid extracts by oral route, in order to see the potential modulation on aminopyrine N-demethylation, UDP-glucuronic acid conjugation and glutathione conjugation. Furthermore, the antioxidant properties such as total phenolics content and total flavonoids content were also assessed to see the involvement of these phytochemicals on modulating drug metabolizing enzymes.

CHAPTER TWO

LITERATURE REVIEW

2.1 Herbal Medicines

Recently, herbal medicines have been gaining popularity worldwide as food supplements and to treat illnesses as they are perceived to be safer and more natural than allopathic medicines (Lynch & Berry, 2007). Accordingly, it is estimated that 60% of the world's population use herbal medicines entirely for medication (Farnsworth, 1994). Besides, the herbal medicines industry in Malaysia is growing at more than 15-20% per year, faster than the general economy. Indeed, annual sales for traditional herbal medicines had increased from USD385 million to USD1.29 billion from 2000 to 2005 and is expected to be worth USD2.5 billion by 2010 (Aziz & Tey, 2009). Herbs by WHO (2000) definition are crude plant materials that include leaves, flowers, fruit, seed, stems, wood, bark, roots, rhizomes or other plant parts which might be completely fragmented or powdered. Conversely, herbal medicines are defined as a plant-derived material or preparation with therapeutic or other human health benefits which contains either raw or processed ingredients from one or more plants (WHO, 2000). The documented early usage of herbal medicines to treat illnesses can be traced back 5000 years ago during Sumerian era, however, archeological evidence suggest even earlier usage of herbal medicines (Raskin, et al., 2002). Additionally, traditional use of herbal medicines might refer to the long historical use with well establish and widely acknowledged safety and effectiveness, and may be accepted by national authorities (WHO, 2000). The systems to utilize traditional herbal medicines vary rather than

uniform across different countries or cultures due to the localized philosophy, religion and the availability of the medicinal herbs in a specific region. This is exemplified by the ancient Traditional Chinese Medicines (TCM) originated from China, 'ayurveda' from India and 'jamu' from Indonesia. Herbal medicines have been demonstrated to protect against many diseases such as diabetes mellitus, cancer, neurodegenerative, gastric, ulcers, ischemic reperfusion, arthritis and inflammatory diseases (Arancibia-Avila, et al., 2008). These beneficial effects have been attributed to the various antioxidants such as polyphenols, ascorbic acid, carotenoids, and tocopherols which are present in the herbal medicines (Du, Li, Ma, & Liang, 2009). In addition, polyphenols have powerful antioxidant activities by scavenging a wide range of reactive species, including hydroxyl radicals, peroxyl radicals, hypochlorous acid and superoxide radical (Harnafi & Amrani, 2008).

There are estimated at least 300,000 species of higher plants worldwide and approximately 10,000 of this plants have a documented medicinal purpose (McChesney, Venkataraman, & Henri, 2007). Thirty five thousands of the species can be encountered in South East Asia and out of 8,000 species are found in Malaysia. The above data suggest that a vast number of plants biodiversity have yet to be explored for its medicinal values. In Malaysia, herbal medicines that have been used for generations for various health benefits are *Eurycoma longifolia*, *Orthosiphon stamineus*, *Andrographis paniculata* and *Centella asiatica*. These medicinal herbs have undergone extensive studies either locally or internationally for its medicinal benefits and will be reviewed further in this chapter.

Eurycoma longifolia is a traditional herb popular in Malaysia and other Southeast Asian countries for its aphrodisiac properties. The plant is called as 'Malaysian ginseng' whereby a tea prepared by cooking 20–50 g of roots for about half an hour and taken as a health tonic and anti-stress remedy (Zanoli, Zavatti, Montanari, & Baraldi, 2009). Traditionally known as 'tongkat Ali', a decoction of the roots is drunk to enhance the virility and sexual prowess (Ong & Nordiana, 1999). It also can be consumed on a daily basis for preventing or treating erectile dysfunction in men (Low & Tan, 2007). The ability of the root extract to modulate sexual behaviour could be ascribed to its testosterone enhancing property (Ang & Lee, 2002). Zanoli et al. (2009) have recently demonstrated that the oral administration of the root powder was able to improve sexual performance in sluggish rats and partially restore the normal sexual behaviour in impotent rats. Besides its main indication for enhancing sexual health, *E. longifolia* also have been proven scientifically to show antimalarial activity (Ang, Chan, & Mak, 1995). Indeed, before scientific evidence proved that *E. longifolia* posses' antimalarial activity, Malaysian aborigines (orang asli) have been consuming *E. longifolia* extract once they have febrifuge and malaria (Ang, et al., 1995). Several active compounds responsible for the aphrodisiac and antimalarial properties such as eurycomanone, eurycomanol and eurycomalactone have been isolated from this plant (Chan, Lee, Sam, & Han, 1989).

Orthosiphon stamineus, known as "misai kucing" in Malaysia and as 'Java tea' in Indonesia, is one of the most popular medicinal plants in Southeast Asia in treating various forms of illnesses including kidney stone and other urinary tract diseases, diabetes mellitus, hypertension, tonsillitis, arteriosclerosis, rheumatism and menstrual

disorders (Awale, Tezuka, Banskota, Adnyana, & Kadota, 2003a; Awale, Tezuka, Banskota, & Kadota, 2003b; Awale, et al., 2001; Tezuka, et al., 2000). It has been introduced into the Western countries since early 20th century (Pan, et al., 2011). In Malaysia, it is mainly use to treat stone disease and gout while in Indonesia it is mainly used as diuretics (Arafat, et al., 2008). There are four main polyphenolic compounds that have been determined in *O. stamineus* namely rosmarinic acid, caffeic acid, sinensetin and eupatorin (Tezuka, et al., 2000). These polyphenolic compounds and caffeic acid are belived to responsible in the diuretic and uricosuric effects in rats (Olah, Radu, Mogosan, Hanganu, & Gocan, 2003). Besides, the methanolic extract of this plant have shown the inhibitory activity on nitric oxide production in macrophage like cells (Awale, et al., 2003a; Awale, et al., 2003b).

Andrographis paniculata, also known as ‘‘king of bitters” due to its extremely bitter taste is a medicinal herb widely distributed in South India, Sri Lanka, China and Malaysia (Lii, Tsai, Yang, & Chen, 2008). In Malaysia it is known as ‘hempedu bumi’ while in Indonesia it is called ‘sambiroto’. It is traditionally employed for centuries in Asia as a folklore remedy for a wide spectrum of ailments. In Scandinavia, an extract of *A. paniculata* called ‘kan jang’, has been used extensively for the past 20 years for the treatment of the common cold (Pekthong, et al., 2008). The herb has been shown to have various pharmacological activities, including anti-inflammatory (Sheeja, Shihab, & Kuttan, 2006), anticancer (Cheung, et al., 2005), immuno-stimulatory (Iruretagoneya, et al., 2005), antiviral (Wiart, et al., 2005), hepatoprotective (Singha, Roy, & Dey, 2007) and antidiabetic (Reyes, et al., 2006). The main active phytochemical in *A. paniculata* is

the diterpene lactone, andrographolide (Zhang & Tan, 1997). In recent times, Chandrasekaran et al. (2010) have reported that the extract of *A. paniculata* containing at least seven phytochemical constituents with significant anti-inflammatory and anti-allergic properties in the animal models investigated. This study provides a rationale for its applications in traditional medicine as anti-inflammatory and antipyretic drug (Chandrasekaran, Gupta, & Agarwal, 2010).

Centella asiatica also known as 'pegaga' in Malaysia is an herb that is commonly eaten fresh as a vegetable (salad), especially among the Malay communities (Hamid, Shah, Muse, & Mohamed, 2002). It is also blended into a drink and used as a cooling drink. This medicinal herb is native to countries like Sri Lanka, Madagascar, South Africa and Malaysia. Fresh extracts of this plant have been used by the people of Java and the Malay Peninsula for many years, as both topical and internal agents for healing of wounds (Hamid, et al., 2002). In an Indian system of medicine, 'ayurveda', this plant is used in the management of central nervous system, skin and gastrointestinal disorder (Subathra, Shila, Devi, & Panneerselvam, 2005). A number of studies have demonstrated the effectiveness of *C. asiatica* triterpenes, in particular the glycoside asiaticoside, in promoting wound healing (Macquart, et al., 1999). The medicinal properties of *C. asiatica* have been ascribed to three triterpenoids: asiatic acid, asiaticoside and madecassic acid (Inamdar, Yeole, Ghogare, & de Souza, 1996). Asiatic acid has been showed to induce apoptosis and cell cycle arrest in different types of cancer (Park, et al., 2007). On the other hand, asiaticoside shows promising wound healing activity in normal as well as in diabetic animals and warrants more detailed

experimental and clinical studies (Wijeweera, Arnason, Koszyckib, & Merali, 2006). It also provides a rationale for the use of *C. asiatica* preparations in the Indian traditional system of medicine to promote wound healing (Shukla, et al., 1999).

Malaysia is blessed with an abundance of various medicinal plants and Malaysia is among the world's 12 mega biodiversity-rich countries, in terms of number of plant species (Institute for Medical Research, 2002). Despite the current preoccupation with combinatorial and chemical synthesis as a vehicle to discover and develop new drugs, the contributions of plant-derived natural product for curing, treating and preventing diseases are still large (Simmond, 2003). Herbs as non-mobile organism produce a large number of secondary metabolites that serve to repel or discourage the use of the plant by insects, microorganisms, animals and men. These secondary metabolites with complex chemical and highly varied structures which are unlikely and uneconomical to be synthesized in the laboratories might be used for new drugs discovery and development (Patrick, 2001). Indeed, herbs have been natural combinational chemist for unimaginable decades and have been selecting products from that combinational library that suitable for interacting specifically with biological target molecules, for examples, membrane receptor or an active site of enzymes (McChesney, et al., 2007). This is emphasized by the fact that 25% of the total numbers of clinically used drugs were derived from plants and this is include the classical drugs atropine from *Atropa belladonna*, codeine and morphine (*Papaver somniferum*), digoxin (*Digitalis* spp.), and quinine (*Cinchona* spp.) (Phillipson, 2007). Herbs therefore remain as promising sources of new drugs in drug discovery and development research and will continue to be so. One successful example

of a clinical drug that derived from plant with sales worth more than USD150 million in 1993 (McChesney, et al., 2007) is paclitaxel (Taxol®) from the bark of mature trees *Taxus brevifolia*. Historically, a journey of paclitaxel was started in 1962 through the National Cancer Institute (NCI) program for evaluation of plant preparations for anticancer activity. The *T. brevifolia* bark extract exhibited a strong cytotoxic activity on *in vitro* cancer cells in 1964 (McChesney, et al., 2007). In 1971, the structure of paclitaxel was elucidated (Wani, Taylor, Wall, Coggon, & McPhail, 1971) and in 1977, extensive studies on animals' model of cancer were conducted and the positive outcomes lead paclitaxel to enter clinical trial. Finally, in 1992 paclitaxel was approved for utilization in the treatment of refractory ovarian cancer. Besides as sources for new drugs, natural product from herb could also act as a template for development of new chemicals entity with enhanced efficacy, high potentcy, less side effect and perhaps distinct pharmacological activity from the parent compound. Other successful drugs candidates that derived from herbs and currently use clinically are anticancer drug, vincristine from *Catharantus roseus*, antimalarial drug, artemisinin (*Artemisia annua*) and ibogaine (*Tabernanthe iboga*) for opiate addiction treatment.

2.2 *Mitragyna speciosa*

2.2.1 Plant Description

Mitragyna speciosa Korth (*M. speciosa*) is found in tropical and subtropical regions of Asia and is categorized in family Rubiaceae. *M. speciosa* is an evergreen and non-seasonal plant that is arborial in character and may grow to a height of 30 meters (Shellard, 1974). This species grows heavily in damp areas rich with humus and is

sensitive towards drought and extreme cold (Shellard, 1974). *M. speciosa* can be characterized by the globular yellow flowering head each containing up to 120 florets (Shellard, 1974). The genus was given the name *Mitragyna* by Korthals because the shape of the stigmas in the species he examined resembled a bishop's mitre (Shellard, 1974). Two types of *M. speciosa* can be distinguished based on the colour of veins in the dark green leaf, either red or green (Chittrakarn, Keawpradub, Sawangjaroen, Kansenalak, & Janchawee, 2010). In Thailand, *M. speciosa* is normally known as 'kratom', 'kakuam', 'ithang' and 'thom' (Suwanlert, 1975). It is a tree native to Malaysia where it is mostly called 'ketum' or 'biak-biak'. In Malaysia, they can be found particularly in northern states of peninsular Malaysia and Selangor (Burkill, 1935; Houghton & Said, 1986).

Figure 2.1: *Mitragyna speciosa* Flower and Leaf. (Image adapted from http://img.alibaba.com/photo/105022189/Kratom_mitragyna_speciosa_red_vein_super_kratom_medicine.jpg. Assessed on 14th April 2011.)

2.2.2 Traditional Applications

Mitragyna speciosa has been employed as an herbal medicine by natives in Southeast Asia particularly in Malaysia and Thailand for decades; however, the exact time for it to begin serving as an herbal medicine cannot be dated. Main traditional application of *M. speciosa* is to wean opium addiction and as a substitute for opium when opium is unavailable (Reanmongkol, Keawpradub, & Sawangjaroen, 2007; Suwanlert, 1975; Tsuchiya, et al., 2002). Additionally, it is often used in its own right as a narcotic drug

(Chittrakarn, et al., 2010). Burkill (1935) reported that Thai people, mainly labourer and farmer, have been using *M. speciosa* to give them a pleasurable effect since it has coca-like stimulant ability at lower dose to combat fatigue and enhance tolerance for hard work under intense sunlight and has opium-like properties at higher dose (Grewal, 1932; Suwanlert, 1975). There is an observation in 1836 that Malay people consumed *M. speciosa* as an opium substitute (Jansen & Prast, 1988). Besides being used as a tonic to boost energy, it is often used to treat diarrhea or intestinal infections by amoeba and protozoa (Chuakul, Temsiririrkkul, Saralamp, & Paonil, 1995). A small number of *M. speciosa* users consumed it as a tonic to prolong sexual intercourse. Other traditional applications of *M. speciosa* as an alternative treatment is to alleviate and treat pain, muscle ache and fatigue, hypertension, cough, fever, malaria, worm infestation and diabetes (Chan, Pakiam, & Rahim, 2005; Jansen & Prast, 1988; Shellard, 1974).

2.2.3 Phytochemistry of *Mitragyna speciosa*

Phytochemistry studies of the constituents of *Mitragyna speciosa* have been reported and over 22 alkaloids have been isolated from *M. speciosa* leaves (Shellard, 1974). Mitragynine, an indole alkaloid was found to be the major constituent of *M. speciosa* leaves, accounting for about half of the total alkaloid contents (Sukrong, et al., 2007) and is believed to contribute to the pharmacological effects of *M. speciosa*. Historically, mitragynine was firstly isolated in 1907 by Hooper and was repeated by Field in 1921 who named the alkaloid mitragynine (Shellard, 1974). The structure of mitragynine was determined with X-ray crystallography by Zacharias, Rosenstein and Jeffrey in 1964 (Shellard, 1974). Chemically known as 9-methoxy-corynantheidine ($C_{23}H_2ON_2O_4$) with

molecular weight of 398.50, mitragynine is soluble in chloroform, alcohol and acetic acid. Its structure resembles yohimbine (Babu, Mccurdy, & Boyer, 2008) and is very stable with a melting point between 102 and 106°C and a boiling point between 230 and 240°C. It absorbs maximally in UV range at 254 nm (Chee, Amirul, Muhammad, Majid, & Mansor, 2008). An average weight of *M. speciosa* leaf is about 1.7 g and a dry leaf is about 0.43 g, therefore, twenty leaves of *M. speciosa* contain approximately 17 mg of mitragynine (Suwanlert, 1975). However, the content of mitragynine varies between different geography locations. This is exemplified by differences in mitragynine content between *M. speciosa* from Malaysia and *M. speciosa* from Thailand whereby *M. speciosa* leaves from adult plants in Thailand have been reported to contain approximately over 60% mitragynine whereas those from Malaysia only contain over 10% (Philipp, Wissenbach, Weber, Zapp, & Maurer, 2010).

Besides mitragynine as the main alkaloid, other indole and oxindole alkaloids are also present in *M. speciosa* leaves. Paynantheine is the second most abundant alkaloid followed by speciogynine and speciociliatine (Takayama, 2004). Mitragynine and paynanthine appear to be exclusive to *M. speciosa*. It appears that mitragynine, speciogynine, paynantheine with small amounts of speciociliatine are present in the leaves of *M. speciosa* (Shellard, 1974). Takayama (2004) has isolated new alkaloid 7-hydroxymitragynine which shows profound pharmacological activity than mitragynine. Other minor alkaloids present in *M. speciosa* are corynantheidaline, corynantheidalinic acid, isopaynantheine, mitragynaline, mitragynalinic acid, mitraciliatine, mitraphylline,

rhynchophylline, speciofoline, and stipulatine (Chittrakarn, et al., 2010; Houghton, Latiff, & Said, 1991; Philipp, et al., 2010; Suwanlert, 1975).

Report on the presence of phenolic compounds in *M. speciosa* is scanty. Only one group in US recently reported that they managed to isolate epicatechin, a flavonoids from *M. speciosa* leaves (León, et al., 2009). There is also not much report on phenolic compounds determined in other species of *Mitragyna* genus. *M. rotundifolia* leaves has been proved to contain quite a number of phenolic compounds and the phenolic compounds were successfully isolated. The phenolic compounds that have been isolated from *M. rotundifolia* leaves were 3,4-dihydroxybenzoic acid, caffeic acid, kaempferol, 4'-*O*-methyl-gallocatechin, catechin and epicatechin (Kang, Li, & Liu, 2010). On the other hand, chlorogenic acid and quercetin were reported to be isolated from the stem bark and roots of *M. inermis* (Asase, et al., 2008) while scopoletic was isolated from *M. parvifolia* (Gupta, Kumar, Bansal, & Singh, 2009).

2.2.4 Pharmacological Activities of *Mitragyna speciosa*

Many studies have been conducted on *Mitragyna speciosa* leaves extracts, mitragynine and its derivatives for the past 44 years due to its potential pharmacologic effects in alleviating pain and weaning opioid addiction (Suwanlert, 1975). Suwanlert (1975) reported that the chronic exposure to *M. speciosa* elicits withdrawal symptoms in humans. The symptoms include irritability, yawning, rhinorrhea, myalgias, diarrhea and arthralgias (Suwanlert, 1975). Additionally, the developments of hyperpigmentation over the cheeks, anorexia, weight loss and psychosis have been described in long-term

M. speciosa addicts (Suwanlert, 1975). Also, chronic users of *M. speciosa* will develop tolerance, a property similar to opiates such as morphine (Pasternak, 2001; Suwanlert, 1975). Existing consistent descriptions of clinical effects of *M. speciosa* stated that it is dose-dependent in effect; stimulant effects at lower doses, and opiate effects predominating at higher doses in humans (Grewal, 1932; Suwanlert, 1975). In *in vivo* experiment, the antinociceptive effect of *M. speciosa* crude extract was more potent than its principle alkaloid alone, mitragynine (Watanabe, Yano, Horie, & Yamamoto, 1997). This finding indicates that minor constituents of *M. speciosa* other than mitragynine may have a very potent antinociceptive effect (Matsumoto, et al., 2004 ; Matsumoto, et al., 2005). The minor constituent has been shown to be 7-hydroxymitragynine (Takayama, 2004). Chittrakarn et al. (2010) reported that methanolic extract of *M. speciosa* leaf and a major alkaloid, mitragynine produced skeletal muscle relaxation with a synergistic effect of pancuronium and succinylcholine. Additionally, its mechanism of action was not only by a competitive antagonism of acetylcholine binding but also had a direct effect on skeletal muscle by decreasing the muscle twitch. *M. speciosa* extract has been demonstrated to stimulate Fos expression in the dorsal raphe nucleus in rats (Kumarnsit, Vongvatcharanon, Keawpradub, & Intasaro, 2007). Fos is a protein product of the proto-oncogene *c-fos* and is used as a marker of neuronal activation (Rodella, Rezzani, Gioia, Tredici, & Bianchi, 1998). *M. speciosa* extract had an antidiarrheal effect by inhibiting diarrheal frequency, total diarrheal score and fecal weight. It also decreased intestinal transit (Chittrakarn, Sawangjaroen, Prasettho, Janchawee, & Keawpradu, 2008). This observation proves the utilization of *M. speciosa* to treat diarrhea in folk medicine. Kumarnsit et al. (2006) has proved that the presumed hypoglycemic activity of *M.*

speciosa extract is not true. They summarized that the alkaloid extract of *M. speciosa* suppresses food and water intakes and slows weight gaining in rats and suggested that the suppressing effects of *M. speciosa* extract on food consumption might be an indirect mechanism that reduces the blood glucose level. *M. speciosa* aqueous and alkaloid extracts also have shown antidepressant-like effects in mouse models of behavioral despair tests. The study indicates that *M. speciosa* leaves extracts have potential to be used in a clinical setting (Kumarnsit, Keawpradub, & Nuankaew, 2007).

Pharmacological effects of *M. speciosa* extract are largely due to the main principle alkaloid, mitragynine. A study by a group of researchers in Japan has proved that mitragynine acts mainly on mu and delta opioid receptor in *in vitro* and *in vivo* studies and possess analgesic effect (Takayama, et al., 2002; Yamamoto, et al., 1999).

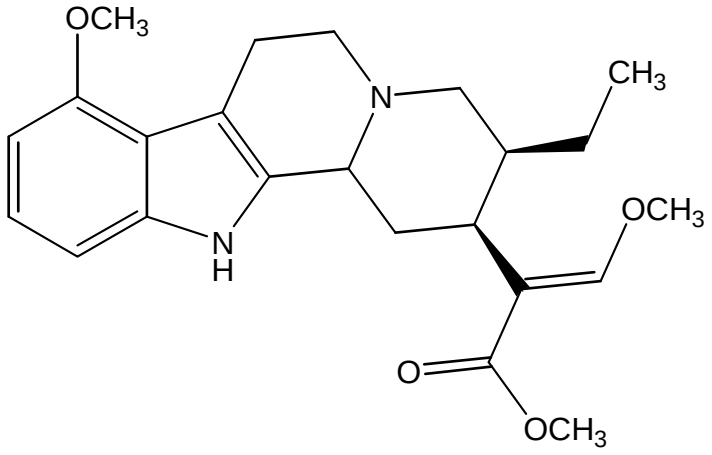

Figure 2.2: Mitragynine Structure.

Mitragynine has been demonstrated to produce an antinociceptive effect through an action on supraspinal opioid receptor and descending noradrenergic and serotonergic systems which are different from morphine mechanism of action (Matsumoto, et al., 1996; Thongpradichote, et al., 1998). Additionally, animal studies suggest that mitragynine may stimulate post-synaptic α_2 adrenergic receptors, and/or block stimulation of 5-HT_{2A} receptors (Matsumoto, et al., 2005b). Mitragynine also has been reported to exert morphine like action on gastric acid secretion in anesthesized rats and inhibits the vas deferens contraction of guinea pig elicited by nerve stimulation (Matsumoto, et al., 2005). Side effects of *M. speciosa* consumption such as anorexia and weight loss have been proved to be mediated by mitragynine through the stimulation of opioid receptors. Like morphine, stimulation of opioid receptors by mitragynine has led to inhibition of 2-deoxy-D-glucose-stimulated gastric acid secretion in urethane-anesthetized rats (Tsuchiya, et al., 2002). Chronic administration of mitragynine significantly reduced the discrimination ratio time on object placement task where it shows mitragynine has impaired the cognitive function (Apryani, Hidayat, Moklas, Fakurazi, & Idayu, 2010). Idayu et al. (2011) have shown that administration of mitragynine is able to produce an obvious antidepressant-like effect in forced swim test and tail suspension test, which is due to interaction with neuroendocrine hypothalamic-pituitary-adrenal axis systems (Idayu, et al., 2011). It was found that the methoxy functional group at C9 of mitragynine controls the maximum activity on opioid receptors (Matsumoto, et al., 2006; Takayama, et al., 2002). A transformation of the methoxy group with other chemicals group at the C9 position on mitragynine has been shown to drastically shift the opioid agonistic activities of mitragynine from a full

agonist to an antagonist of opioid receptors (Matsumoto, et al., 2006). Mitragynine related compounds also express interesting opioid activities especially 7-hydroxymitragynine and mitragynine pseudoindoxyl. They are found to exhibit potent antinociceptive action trough the same mechanism as mitragynine via interaction with mu and delta opioid receptors (Matsumoto, et al., 1996). In fact, the 7-hydroxymitragynine exhibited about 13 times higher potency than morphine and about 46 times higher potency than mitragynine in animal studies (Matsumoto et al., 2004). On the other hand, mitragynine pseudoindoxy interacted with mu opioid receptor about 100 and 20 folds higher than that of mitragynine and morphine respectively (Takayama, Aimi, & Sakai, 2000). It is interesting to bring out that a nitrogen atom, a benzene residue, and an oxygen atom, on the benzene ring in the structures of morphine and 7-hydroxymitragynine could not superimpose using molecular modeling techniques (Matsumoto, et al., 2005a). The aforementioned functional groups play an important role in producing analgesic activity (Dhawan, et al., 1996). For that reason, it is speculated that 7-hydroxymitragynine binds opioid receptor sites other than those that morphine binds (Matsumoto, et al., 2008). Due to its unique structure and a potent activity, 7-hydroxymitragynine may be used as a template for development of novel analgesics with distinct mechanisms from morphine.

2.2.5 *Mitragyna speciosa* Legal Status

Mitragyna speciosa leaves have been abused by drug addicts since some alkaloids (mainly mitragynine) from the plant posses opiate and cocaine like effects (Matsumoto, et al., 2004). To date, *M. speciosa* uses has been banned in Malaysia, Thailand,

Myanmar, Vietnam, Bhutan, Finland, Poland, Lithuania, Denmark and Australia due to its highly misuse potential. *M. speciosa* has been prohibited in Thailand since 1943 whereby existing trees require to be cut down and it is illegal to buy, sell, import, growing and harvesting *M. speciosa*. Furthermore, Thailand is the only country in the world that classified *M. speciosa* in Category V of a five category classification of narcotics under Thai government Narcotics Act B.E. 2522, placing *M. speciosa* along with marijuana (Chittrakarn, et al., 2010). This implies the seriousness of *M. speciosa* misuse in Thailand and problems that may arise from its consumption. In Malaysia, *M. speciosa* was listed in the First Schedule and the Third Schedule (psychotropic substances) of the Poisons Act 1952 in August 2004 (Chan, et al., 2005). Once this act was enacted, individuals who possess or sell *M. speciosa* leaves or other *M. speciosa* preparations such as drinks and teas containing mitragynine will need to pay a penalty of RM 10,000, a four-year jail sentence or both (Chan, et al., 2005). More recently, the act is reported to be revised so that *M. speciosa* will be listed as a dangerous drug thus making the selling of it a serious drug offence. The enforcement of this law in Malaysia is necessary due to the escalating demand of *M. speciosa* extract (as concoction or tea) since the year 2000 as a cheap and easily available alternative of other drugs such as cannabis and heroin. Furthermore, there is a perception that the consumption of *M. speciosa* leads to the abuse of other drugs such as cannabis and heroin (Chan, et al., 2005). On the contrary, *M. speciosa* is currently not illegal in most of the European Countries and in the USA. As a consequence, there is a high demand on this plant in these countries as a substitute for other illegal drugs (Babu, et al., 2008). Moreover, *M.*

speciosa leaves powder can be purchased online at a wide variety of shops online with low price (Chittrakarn, et al., 2008).

2.3 Drug Metabolizing Enzymes

Xenobiotics by definition are substances that are foreign and not nutrients for human body, which can enter human body through ingestion, inhalation or absorption (Brahmankar & Jaiswal, 1995). Once xenobiotics enter the body, they need to be excreted or they accumulate in the body and precipitate toxicity. On the other hand, drugs are any chemical substances that affect the structure or function of a living organism that are widely used for the prevention, diagnosis and treatment of diseases and for the relief of symptoms (Martin, 2007). Therefore, drugs can be regarded as xenobiotics that are not nutrients for the body and must be eliminated once they had elicited therapeutic effect or it will be accumulated and become toxic to human. There are two ways of drugs elimination from the body namely renal excretion and metabolism (Correia, 2004). Renal excretion plays an important role in eliminating biologically active drugs through glomerular filtration, only if the drugs are sufficiently water soluble. However, this is not the case of most therapeutic drugs which need to be adequately lipid soluble so that it can be absorbed effectively from the gastrointestinal tract to systemic circulation when taken orally. This physicochemical property hinders the excretion of drugs via glomerular filtration since the lipophilic nature of renal membranes will reabsorb the drugs into the systemic circulation. Fortunately, the human body is armed with a set of enzymes specialized to metabolize drugs by addition of functional group and endogenous molecules to the drug, hence it become more polar and

can be excreted via the kidney. Therefore, metabolism of drugs is a chemical process that transform chemical from one form to another so that it can be eliminated from human body (Brahmankar & Jaiswal, 1995). Drug metabolizing enzymes were traditionally grouped into phase 1 and phase II based on the reaction it catalyzes (Williams, 1972). Phase I group of enzymes catalyze functionalization reactions by introducing, modifying or unmasking functional group such as hydroxyl (OH), amino (NH) and carboxylic acid (COOH). If phase I products are polar enough, they can directly be excreted via kidney. However, most drugs are not polar enough eventhough after the addition of functional groups. Therefore, phase I reaction products need to undergo another reaction called phase II reactions. In phase II reactions, phase II group of enzymes catalyze conjugation reactions by adding polar endogenous molecules such glucuronic acid, amino acids and glutathione which enhance the polarity of the phase I drug metabolites. Conjugation reactions generally result in products with total loss of pharmacologic activity and high polarity, hence, are better known as true detoxification reactions (Brahmankar & Jaiswal, 1995).

Liver is the main site of drug metabolizing enzymes and it is strategically located between the portal vein which transports blood from the gastrointestinal tract (GIT) to the liver and the inferior vena cava which drains blood from liver to the heart for circulation to the whole body (Holt & Smith, 2008). Other organs are also capable to metabolize drugs to certain extent particularly lungs, kidneys, intestine, placenta, adrenals and skins (Brahmankar & Jaiswal, 1995). At the subcellular level, drug metabolizing enzymes are mainly located in the smooth endoplasmic reticulum and

cytosol (Correia, 2004). Route of administration of most drugs are by oral since it is the most convenience and easiest way (Sastry, Nyshadham, & Fix, 2000). Following oral administration, drugs are absorbed from the GIT to the liver via portal vein where they will be metabolized extensively. This process has been called as a first pass metabolism effect (Correia, 2004). Due to extensive metabolism in the liver, only a small portion of parent drug molecules can reach the blood circulation whereas the rest are in the form of drug metabolites. Bioavailability is the amount of parent drugs that reach blood circulation after undergoing a first pass metabolism effect in the liver. Therefore, the amounts of a parent drug that reaches the blood circulation is actually less than the amount taken by oral route because of metabolism make bioavailability less than 100%. Therefore, it could be concluded that, for most drugs, the amount of a particular drug that a human consumed orally is actually more than needed for a therapeutic effect. Nevertheless, the established oral dosages of drugs have undergone extensive research before they were marketed so that only a safe amount of parent drug may reach the blood circulation.

2.3.1 Phase I Drug Metabolizing Enzymes

Phase I drug metabolizing enzymes can catalyze oxidation, reduction and hydrolysis to respectively introduce new polar group, modify an existing functional group and unmask existing polar functional group onto a parent drug (King, 2009). Phase I drug metabolizing enzymes which are involved in oxidation, reduction and hydrolysis reactions are listed in Table 2.1. Oxidation reaction is the most common compared to the

other reactions. Furthermore, oxidation by cytochromes P450 is responsible for the metabolism of 75% of marketed drugs (Williams, et al., 2004).

Table 2.1: Phase I Drug Metabolizing Enzymes which are Involved in Oxidation, Reduction and Hydrolysis (Gibson & Skett, 1994).

Enzyme	Reaction
Cytochromes P450	Oxidation
Alcohol dehydrogenase	Oxidation
Aldehyde dehydrogenase	Oxidation
Xanthine oxidase	Oxidation
Aldo/keto reductase	Reduction
NADPH quinone oxidoreductase	Reduction
Carbonyl reductase	Reduction
Acetylcholinesterase	Hydrolysis
Carboxylesterase	Hydrolysis
Aminopeptidase	Hydrolysis

2.3.1 (a) Cytochrome P450

Cytochrome P450, CYP450 (EC 1.14.14.1) belong to a superfamily of heme-containing enzymes with more than 4000 members (Urlacher & Eiben, 2006). CYP450 derived its name from the peculiar difference spectrophotometric absorbance peak unlike typical hemoprotein at 450 nm when reduced by a reducing agent and bound by carbon monoxide (Omura & Sato, 1964). CYP450 is the primary enzymes catalyzing the oxidations of a variety of endogenous and exogenous chemicals including steroids, drugs, and chemical carcinogens (Guengerich, 1992). Table 2.2 lists the type of oxidation reactions that CYP450 may catalyze for a particular drug. Additionally, CYP450 are responsible for metabolizing almost 75% of top 200 marketed drugs in the USA (Williams, et al., 2004).

Table 2.2: Type of Oxidation Reaction Catalyzed by CYP450 (Gibson & Skett, 1994).

Drug	Type of Oxidation Reaction
Lignocaine	Aromatic hydroxylation
Pentobarbitone	Aliphatic hydroxylation
Diazepam	N-Dealkylation
Codeine	O-Dealkylation
Amphetamine	Oxidative deamination
Chlorpromazine	O-Dealkylation
Halothane	Dehalogenation
Ethanol	Alcohol oxidation

CYP450 introduced functional groups onto drug molecules by insertion of atomic oxygen from molecular oxygen into a substrate with the simultaneous reduction of the other atom to water (Bernhardt, 2006) like the following reaction:

$$RH + O_2 + NADPH + H^+ \rightarrow ROH + H_2O + NADP^+$$

This reaction requires CYP450, NADPH-cytochrome P450 reductase and phospholipids. The system is collectively known as mixed function oxidase or monooxygenase. The reductase is responsible for the transfer of two electrons from NADPH to CYP450. The electrons caused oxidoreduction reaction on CYP450 which split the molecular oxygen. In this case, one oxygen atom will be inserted into drugs and the other oxygen atom is reduced to water (Uetrecht & Trager, 2007).

All CYP450 carry a CYP prefix to show that they are the members of the cytochrome P450 superfamily. Human CYP450 can be grouped into several families depending on the similarity of their gene sequences. Enzymes with more than 40% sequence similarity are assigned to the same family, while those with more than 55% are