THE EFFECTS OF *GYNURA PROCUMBENS* EXTRACTS ON DRUG METABOLIZING ENZYMES

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THE EFFECTS OF *GYNURA PROCUMBENS* EXTRACTS ON DRUG METABOLIZING ENZYMES

by

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LIST OF SYMBOLS

%	Percentage sign
°C	Degree Celsius
µg	Microgram
µg/mL	Microgram per milliliter
µL	Microliter
µM	Micromolar
g	Grams
mg	Milligram
min	Minute
mM	Milimolar
pmol	Picomole
nmole	Nanomole
R^2	Coefficient of determination
v/v	Volume over volume

LIST OF ABBREVIATIONS

$AICI_3$	Aluminium chloride
ANOVA	Analysis of variance
BSA	Bovine serum albumin
CDNB	1-chloro-2,4-dinitrobenzene
$CuSO_4.5H_2O$	Copper(II) sulfat pentahydrate
CYP450:	Cytochrome P450
DPPH	2,2-diphenyl-1-picrylhydrazyl
DSHEA	Dietary Supplement Health and Education Act
DOX	Doxorubicin
EGCG	Epigallocatechin-3-gallate
FDA	Food and Drug Administration
FMO	Flavin monooxygenase
GAE	Gallic acid equivalent
GSH	Glutathione
GST	Glutathione S-transferases
HPLC	High Perfomance Liquid Chromatography
HPTLC	High Perfomance Thin Layer Chromatography
IC_{50}	Half maximal inhibitory concentration
K_i	Inhibitor constant
K_m	Michaelis-Menten constant
Luciferin-BE	Luciferin benzyl ether
Luciferin-ME	Luciferin methyl ether

$MgCl_2$	Magnesium chloride
MMP-1	Matrix metalloproteinase-1
MMP-9	Matrix metallopeptidase 9
MS	Mass spectrophotometry
Na_2CO_3	Sodium carbonate
NADPH	Nicotinamide adenine dinucleotide phosphate hydrogenase
$NADP^+$	Nicotinamide adenine dinucleotide phosphate
NaK Tartrate	Sodium potassium tartrate
$NaNO_2$	Sodium nitrite
NaOH	Sodium hydroxide
NAPQI	N-acetyl-p-benzoquinone imine
NATs	N-acetyltransferases
PAH	Polycyclic aromatic hydrocarbon
*p*NP	Para-nitrophenol
QE	Quercetin equivalent
RLM	Rat liver microsome
RLU	Relative Light Unit
ROS	Reactive oxygen species
SEM	Standard error mean
SULTs	Sulfotransferases
TCA	Trichloroacetic acid
TIM	Traditional Indian Medicine
Tris-HCl	Tris-hydrochloride
UDPGA	Uridine 5’-diphospho-glucuronic acid

UGT	Uridine-diphospho-glucuronosyl transferases
US	United States
V_{max}	Maximal reaction velocity
WHO	World Health Organization

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KESAN EKSTRAK *GYNURA PROCUMBENS* TERHADAP ENZIM METABOLISME DRUG

ABSTRAK

Kebangkitan penggunaan ubat-ubatan herba di seluruh dunia, dan penggunaan bersama drug konvensional dan terapi tradisional telah menjadi kebiasaan. *Gynura procumbens* ialah herba malar hijau yang lazimnya dimakan secara mentah atau diminum sebagai teh di Malaysia. Walaupun pelbagai kajian mengenai aktiviti farmakologi *Gynura procumbens* telah dijalankan, interaksi di antara herba ini dengan enzim metabolisme drug masih tidak diketahui. Ini adalah kajian pertama berkaitan dengan modulasi ekstrak *Gynura procumbens* terhadap enzim metabolisme drug. Pengekstrakan daun *Gynura procumbens* dengan air, etanol dan metanol masing-masing menghasilkan peratusan hasil sebanyak 27.50%, 7.80% dan 4.20%. Kuantifikasi dua sebatian penanda kaempferol-3-O-rutinosida dan astragalin di dalam setiap ekstrak adalah berjaya kecuali ekstrak akueus *Gynura procumbens.* Setiap ekstrak etanol dan metanol masing-masing mengandungi kaempferol-3-O-rutinosida dan astagalin sebanyak 1.60% dan 1.79% dan 2.33% dan 3.83%. Bagaimanapun, kaempferol-3-O-rutinosida dan astragalin tidak dapat dikenal pasti di dalam ekstrak akueus. Kandungan sebatian fenolik di dalam ekstrak *Gynura procumbens* boleh disenaraikan dalam turutan menurun seperti berikut: metanol > etanol > akueus, manakala kandungan sebatian flavonoid di dalam ekstrak *Gynura procumbens* boleh disenaraikan dalam turutan menurun seperti etanol >metanol > akueus. Ekstrak metanol menunjukkan penghapus radikal bebas yang paling aktif berbanding ekstrak lain.

Ekstrak etanol menunjukkan kesan perencatan yang kuat terhadap enzim CYP3A4, CYP1A2 dan GST masing-masing dengan nilai IC_{50} 32.01 ± 1.11 μg/mL, 7.87 ± 1.22 μg/mL dan 44.62 ± 1.12 μg/mL. Bagaimanapun, ekstrak etanol tidak menunjukan perencatan yang ketara terhadap enzim UGT. Ekstrak methanol menunjukkan kesan perencatan yang lemah terhadap enzim CYP3A4, CYP1A2 dan GST masing-masing dengan nilai IC_{50} lebih daripada 100 μg/mL. Ekstrak metanol juga tidak menjejaskan enzim UGT. Ekstrak akueus sebaliknya, tidak menunjukkan kesan perencatan terhadap semua enzim yang dikaji. Kesimpulannya, perencatan enzim metabolisme drug mengikut turutan kandungan sebatian flavonoid (ekstrak etanol > ekstrak metanol > ekstrak akueus) iaitu semakin tinggi kandungan sebatian flavonoid, semakin kuat perencatan enzim metabolisme drug.

THE EFFECTS OF *GYNURA PROCUMBENS* EXTRACTS ON DRUG METABOLIZING ENZYMES

ABSTRACT

Resurgence in the use of herbal medicines worldwide and the co-use of conventional drug and traditional therapies is becoming more common. *Gynura procumbens* is an evergreen herb that has been commonly eaten raw or drink as tea in Malaysia. Despite various studies conducted on the pharmacological activities of *Gynura procumbens*, the interaction between this herb with drug metabolizing enzymes is still unknown. This is the first study regarding the modulation of *Gynura procumbens* extracts towards drug metabolizing enzymes. Extraction of *Gynura procumbens* leaves with water, ethanol and methanol produced a percentage of yields of 27.50%, 7.80% and 4.20% respectively. Quantification of two marker compounds kaempferol-3-O-rutinoside and astragalin in each extracts is successful except for the aqueous extract of *Gynura procumbens*. Each ethanol and methanol extracts contain 1.60% and 1.79% and 2.33% and 3.83% of kaempferol-3-O-rutinoside and astragalin respectively. Kaempferol-3-O-rutinoside and astragalin, however, could not be identified in aqueous extract. The content of phenolic compounds in *Gynura procumbens* extracts can be ranked in decreasing order as methanol extract > ethanol extract > aqueous extract, whereas the content of flavonoid compounds in *Gynura procumbens* extracts can be ranked in decreasing order as follows: ethanol extract > methanol extract > aqueous extract.

The methanol extract of *Gynura procumbens* exhibited the most active free radical scavenger compared to the other extracts. Ethanol extract exhibited strong inhibitory effect on CYP3A4, CYP1A2 and GST enzyme with IC_{50} values of 32.01 ± 1.11μg/mL, 7.87 ± 1.22μg/mL and 44.62 ± 1.12μg/mL respectively. However, ethanol extract did not show significant inhibition on UGT enzymes. Methanol extract exhibited weak inhibitory effect on CYP3A4, CYP1A2 and GST enzymes with IC_{50} values more than 100 μg/mL. Similar to ethanol extract, methanol extract also did not affect UGT enzymes. Aqueous extract, on the other hand, demonstrated no inhibitory effect on all enzymes studied. In conclusion, the inhibition of drug metabolizing enzymes in this study follows the rank order of total flavonoid content (ethanol extract > methanol extract > aqueous extract) in which the higher the total flavonoid content, the stronger the inhibition of drug metabolizing enzymes studied.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Herbal medicine has gained great attention throughout the world. Herbal medicine is defined as a medicine which is made from plants, including seeds, berries, roots, leaves, bark. Herb plants have been used for medicinal treatment as early as 3,000 BC by the ancients Chinese and Egyptians. Africans and native Americans have also used herbs in their healing rituals while the Indians practiced the use of herbs in Ayurveda treatment, which is a system of traditional Indian medicine (TIM) (Patwardhan et al., 2005). In the latest issue of Herbal Gram, American Botanical Council reported herbal supplement sales in all channels reached up to $5.3 billion in the United States in 2011, which is an increase of 4.3% of the total sales in 2010 (Schultz, 2012). Herbal medicine has been receiving continuous overwhelming response from all over the world because it is safe, sustainable, readily available (can be eaten raw) and it is an alternative medicine to conventional drug which is usually expensive. In the year 1994, U.S. Dietary Supplement Health and Education Act (DSHEA) have classified herbal medicine as 'dietary supplement'. As a result, herbal medicines, unlike prescription drug, can be sold without prior safety and effectiveness tests and if the U.S Food and Drug Administration (FDA) prove a particular herbal medicine as harmful and dangerous for human consumption, only then it will be removed from the market (Brent and Bauer, 2003).

There is huge demand for herbal medicinal plants from pharmaceutical companies, phytopharmaceutical companies, health product companies, traditional and alternative practitioners due to their great medical and health benefits. Currently, there are 40 top-selling herbal dietary supplements in the United States and cranberry (*Vaccinium macrocarpon*) is the best-selling herbal product in the year of 2011, followed by, saw palmetto (*Serenoa repens*), soy (*Glycine max*), ginkgo (*Ginkgo biloba*), garlic (*Allium sativum*), milk thistle (*Silybum marianum*) and so forth (Blumenthal et al., 2012). Ginkgo (*Ginkgo biloba)* is believed to heal memory impairment, stroke, edema, inflammation, Alzheimer's disease and vasso oclusive crisis (Diamond et al., 2000) while garlic (*Allium sativum*) is suggested to have antilipidemic, antihypertensive, antiglycemic and antithrombotic properties (Ackermann et al., 2001). Echinacea or purple coneflower (*Echinacea purpurea*) is commonly used to treat influenza and common cold in the United States. Besides that, ginseng (*Panax ginseng*), another traditional herbal plant widely used in the United States is known to enhanced human immune system, increase physical stamina and decrease fatigueness (Foti and Wahlstrom, 2008). Although herbal medicine promises a great deal of benefits health care, a large number of investigations have reported its adverse side effects and possible fatality in some cases. Herbal medicine usually contains a mixture of more than one active ingredient. Interactions between herbal medicine and its corresponding active constituents towards drug metabolizing enzyme are summarized in Table 1.1.

Table 1.1: Interactions between herbal medicines and drug metabolizing enzymes.

Herbal medicines	Scientific Name	Active constituent	Interaction	References
Black cohosh	*Actaea racemosa*	Triterpene glycoside	Inhibit CYP2D6	Gurley et al., 2005
Angelica root	*Angelica dahurica*	Furanocoumarin derivatives	Inhibit CYP2C, 2D1 and 3A	Ishihara et al., 2000
Black pepper	*Piper nigrum*	Alkaloid piperine	Inhibit CYP3A4	Bhardwaj et al., 2002
Grapefruit juice	*Citrus paradisi*	Bergamottin, 6'7'-dihydroxybergamottin	Inhibit 1A2, 2A6, 2C9, 2C19, 2D6, 2E1 and 3A4	He et al., 1998
Kava	*Piper methysticum*	Kavalactones	Inhibit CYP1A, 2C9, 2C19, 3A4 and 2D6	Foster et al., 2003
Milk thistle	*Silybum marianum*	Silymarin	Decrease bilirubin conjugation	Salmi and Sarna, 1982
St. John's wort	*Hypericum perforatum*	Hypericin, hyperforin	Inhibit CYP1A2, 2C9, 2C19, 2D6 and 3A4	Wang et al., 2001
Soy	*Glycine max*	Daidzein, genistein	CYP3A4	Foster et al., 2003
Saw palmetto	*Serenoa repens*	Fatty acid, plant sterols, flavonoids	Inhibit CYP3A4, 2D6,2C9	Yale et al., 2005

Table 1-1. Continued.

Herbal medicines	Scientific Name	Active constituent	Interaction	References
Schisandra fruit	*Schisandra chinensis*	Schisandrin, gomisin	Inhibit CYP3A4	Iwata et al., 2004
Valerian	*Valeriana officinalis*	Valerenic acid, valepotriates, alkaloids, furanofuran lignans, free amino acids	Inhibit 2C19, 2D6 and 3A4	Strandell et al., 2004
Licorice	*Glycyrrhiza glabra*	Glycyrrhizin	Inhibit CYP3A4	Budzinski et al., 2000
Hempedu bumi	*Andrographis paniculata*	Andrographalide	Inhibit UGT2B7	Zainal Abidin et al., 2014
Misai Kucing	*Orthosiphon stamineus*	Sinensetin, eupatorin, rosmarinic acid	Inhibit GST	Tan et al., 2011
Temu lawak	*Curcuma xanthorrhiza*	Curcumin, curcumene, xanthorrhizol	Inhibit UGT1A1 and 2B7	Mohd Salleh., 2015
Kratom	*Mitragyna speciosa*	Mitragynine	Weakly inhibit GST	Azizi et al., 2010
Cranberry	*Vaccinium oxycoccos*	Anthocyanins, flavonols, quercetin	Inhibit CYP3A	Uesawa et al., 2006

For example, grapefruit juice contains two of the most abundant furanocoumarins, namely bergamottin and 6',7'-dihydroxybergamottin, which are known to be responsible for herb-drug interaction (Zhou et al., 2004). Milk thistle (*Silybum marianum*) contains flavonolignans, which are present as multiple structural isomers including silymarin, and flavonolignans have been evalueted to inhibit Phase I drug metabolizing enzyme at low concentration (Foti and Wahlstrom, 2008). Piperine, the active chemical constituent that can be found in *Piper nigrum Linn* and *Piper Longum Linn*, is responsible for raising the concentration of several drugs in blood plasma such as phenytoin (antiepileptic drug), propanolol (drug used to treat high blood pressure) and theophylline (drug used to treat respiratory disease). It is also increased the plasma concentration of rifampicin in patients with pulmonary tuberculosis (Hu et al., 2005). Garlic (*Allium sativum*) is reported to have several of compounds such as allicin and alliin, flavonoids, polysaccharides, prostaglandins, saponins and terpenes. Some reports have stated the co-administration of warfarin with garlic extract increases the clotting time and international normalised ratio and result in spontaneous spinal epidural haematoma and postoperative bleeding (Hu et al., 2005).

Gynura procumbens which is known as Sambung Nyawa in Malaysia is widely distributed in South East Asian countries. *Gynura procumbens* leaves have been used traditionally to treat various diseases. Many researchers reported that this herbal plant exhibited pharmacological potential as anti-diabetic, anti-inflammatory, anti-hypertensive and more (Hassan et al., 2010; Lee et al., 2012; Iskander et al., 2002; Kim et al., 2006).

Despite numerous studies on the various pharmacological effects of *Gynura procumbens*, its inhibitory effects on drug metabolizing enzymes have not been investigated to date. Hence, the present study was carried out to expand the study on the effects of varying *Gynura procumbens* extracts on drug metabolizing enzymes activities. Quantification of two marker compounds using a high performance liquid chromatography (HPLC) method and standardization of *Gynura procumbens* extracts by biological profiling (determination of total phenolic and flavonoid content, and antioxidant activity) were also carried out. The experiments of the present study were summarized in Figure 1.1.

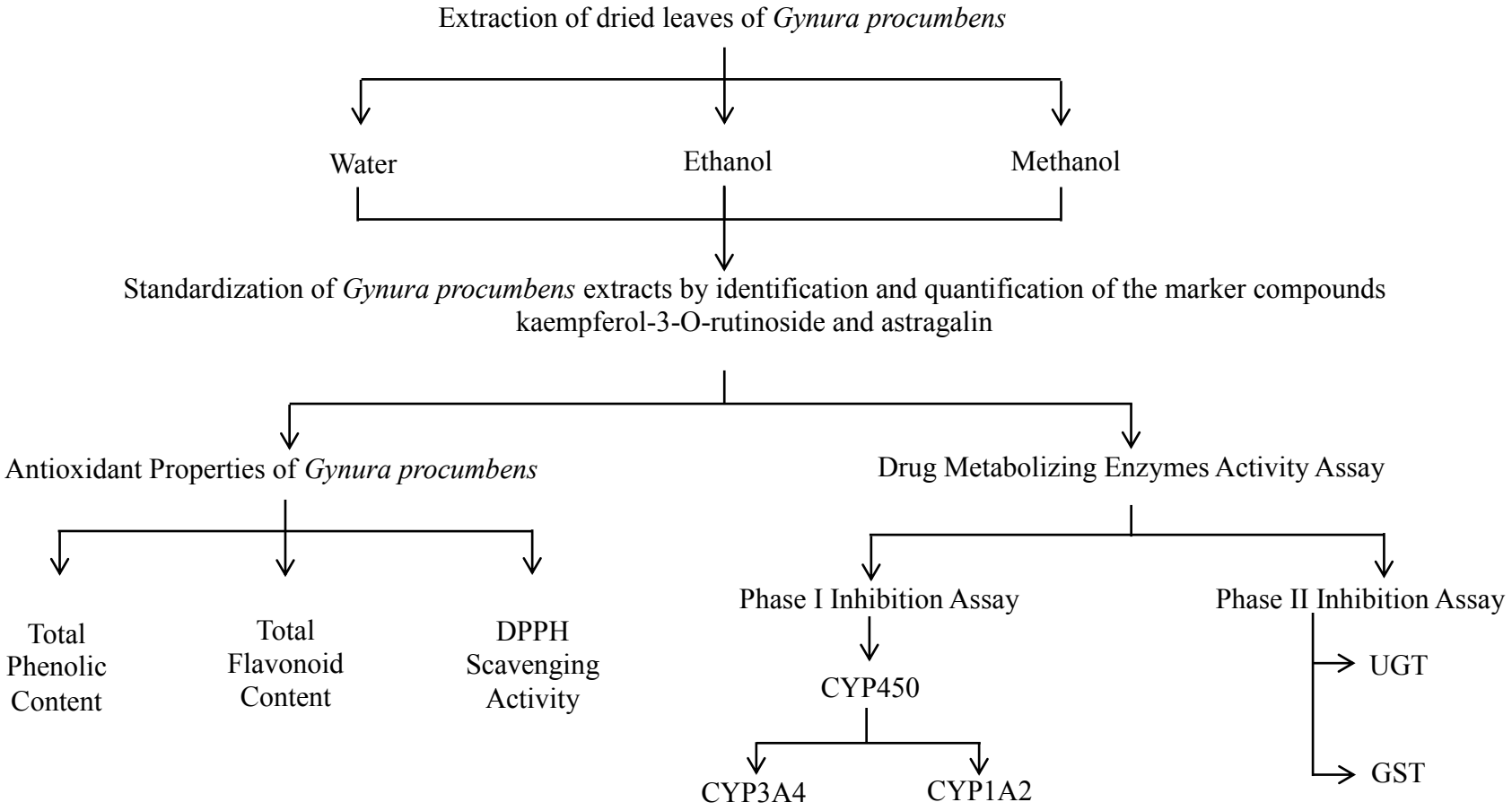


Figure 1.1: The experimental design for the effects of *Gynura procumbens* extracts on drug metabolizing enzymes study.

1.2 The Problem Statement of the Study

Gynura Procumbens have been used traditionally to treat various types of diseases. It is also have been studied extensively by researchers to investigate the pharmacology properties of *Gynura procumbens* so that this plant can be a natural and non-toxic medication for diseases for example in treating diabetes patient. Since *Gynura procumbens* has been eaten or drank for medicinal purposes, it is crucial to investigate the effect of this herbal plant on drug metabolizing enzymes to predict any changes in drug metabolizing enzymes activity which may lead to serious side effects.

1.3 The Purpose of the Study

The aim of the study is to investigate the effects of *Gynura procumbens* extracts on Phase I and Phase II drug metabolizing enzymes.

1.4 The Objectives of the Study

The objectives of the present study are:

1. To quantify the amount of two marker compounds (kaempferol-3-O-rutinoside and astragalin) in each extract of *Gynura procumbens*.
2. To study the total phenolic content, total flavonoid content and DPPH scavenging activity of each extracts of *Gynura procumbens*.
3. To evaluate the effect of *Gynura procumbens* extracts on human recombinant CYP3A4 and CYP1A2 enzymes isoforms respectively by using luciferin derivatives as the marker reaction for CYP3A4 and CYP1A2 enzymes activity.

4. To study the effect of *Gynura procumbens* extract on rat liver microsomes (RLM) UDP-glucuronosyltransferases (UGT) enzyme by employing *p*-nitrophenol (*p*-NP) as the marker reaction for UGT enzyme activity.
5. To study the effect of *Gynura procumbens* extract on rat liver cytosolic fraction glutathione S-transferases (GST) enzyme by employing 1-chloro-2,4-dinitrobenzene (CDNB) as the marker reaction for GST enzyme activity.

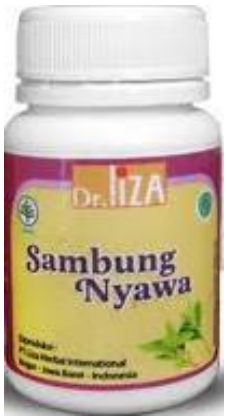
CHAPTER TWO

LITERATURE REVIEWS

2.1 Description of *Gynura procumbens*

Gynura procumbens (Figure 2.1) is an annual evergreen shrub of the Compositae family which can be found in Indonesia, Thailand, and Malaysia. *Gynura procumbens* is locally known in Malaysia as 'Akar Sebiak', 'Kecam Akar' or 'Sambung Nyawa' (Bhore and Vaishana, 2010). It is factual that the leaves of *Gynura procumbens* are not bitter at all and it can be eaten raw as salad. *Gynura procumbens* is mostly used by the Malays in Malaysia as salad. They also can be sun-dried, preserved as tea. The tea can be prepared by steeping the leaves of *Gynura procumbens* in boiled water for about 5 minutes and it is ready to be consumed either hot or cold. Honey or sugar can be added to reduce the bitterness of *Gynura procumbens* tea. *Gynura procumbens* also sold over-the-counter in capsule form especially in Indonesia (Figure 2.1).

Figure 2.1: *Gynura procumbens*

A 

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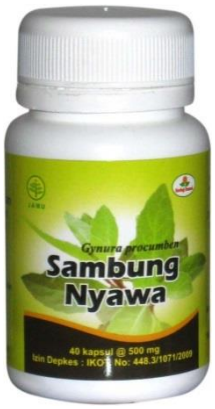
C 

Figure 2.2: Capsule of *Gynura procumbens* sold in the market. Pictures are adopted from panels (a) www.sambungnyawa.com; (b) www.etalasemuslim.com; and (c) www.istanaherbal.com.

2.2 Taxonomy of *Gynura procumbens*

Kingdom : Plantae

Division : Magnoliophyta

Class : Magnoliopsida

Order : Asterales

Family : Asteraceae

Genus *: Gynura*

Species *: Gynura procumbens*

2.3 Pharmacological Potentials of *Gynura procumbens*

Gynura procumbens is traditionally used to treat various types of illnesses such as fever, rash inflammation, kidney disease, hemorrhoids and diabetes mellitus (Kim et al., 2006; Saiman et al., 2012).

The leaves of *Gynura procumbens* are proven to be non-toxic (Yam et al., 2009) and they exhibited anti-diabetic (Hassan et al., 2010; Lee et al., 2012), anti-oxidative (Puangpronpitag et al., 2010), anti-inflammatory (Iskander et al., 2002), and anti-hypertensive characteristics (Kim et al., 2006). Ethanol extract of *Gynura procumbens* may also have biguanide-like activity since it is shown to reduced serum cholesterol and triglyceride levels when an optimum dose was given over a period of a group of diabetic rats (Zhang and Tan, 2000). *Gynura procumbens* has been demonstrated to decrease blood pressure in spontaneously hypertensive rats via the inhibition of the angiotensin-converting enzyme (Hoe et al., 2007).

However, the same author has proposed butanolic fraction of *Gynura procumbens* may contribute to hypotensive effect in rats via other mechanism. The investigation has reported butanolic fraction of *Gynura procumbens* resulted in lowered blood pressure (hypotensive effect) in rats as a result of vasodilation due to inhibition of Ca^{2+} influx via receptor-operated and/or voltage-dependent calcium channel (Hoe et al., 2007). In addition, ethanol extract of *Gynura procumbens* inhibited MMP-1 and MMP-9 expressions which are induced by UVB irradiation via the inhibition of pro-inflammatory cytokine mediator release and reactive oxygen species (ROS) production (Kim et al., 2011).

Ethanol extract of *Gynura procumbens* is also shown to have antiproliferative activity on male rat's livers induced by 7,12-dimethylbenz[α]anthracene (Nisa et al., 2012). According to Nurulita (2012) in her recent study, ethyl acetate fraction of *Gynura procumbens* in combination with doxorubicin (DOX) potentiate DOX effect on breast cancer cell growth inhibition and hence this fraction could be developed as co-chemotherapy agent in reversing multidrug resistance.

2.4 Phytochemical constituents of *Gynura procumbens*

Numerous studies have exposed that *Gynura procumbens* leaves extract contains various active compounds such as flavanoids, saponins, sterol glycoside, terpenoids and tannins (Zahra et al., 2011). Previous studies have demonstrated that *Gynura procumbens* which extracted using various concentration of ethanol as extraction solvent (95%, 75%, 50%, 25%, and 0% of ethanol (% v/v)) with different extraction methods (Soxhlet, maceration and ultra-sonication) showed vary in phenolic content (Algariri et al., 2013).

The total content of phenolic compounds in various extracts of *Gynura procumbens* is ranked in decreasing order as 50% ethanol > 75% ethanol > 95% ethanol > 25% ethanol > 0% ethanol. However, total flavonoid content of *Gynura procumbens* was found to decrease with the increase in polarity of the extraction solvent (Algariri et al., 2013). Crude methanol extract of *Gynura procumbens* and its fractions (chloroform, ethyl acetate, *n*-butanol, and aqueous) had showed different level of antioxidant potential. Ethyl acetate fraction exhibited the highest antioxidant properties compared to the other extracts and fractions.

2.5 Phytochemical Analysis of *Gynura procumbens*

The chemical constituents of *Gynura procumbens* that is responsible for its antioxidant action was determined using HPTLC and it is found that the content of astragalin and kaempferol-3-O-rutinoside were relatively high in the ethyl acetate fraction (Yam et al., 2008). Three components from *Gynura procumbens* ethanol extract (quercetin 3-O-rutinoside, isobioquercetin and kaempferol 3-O-rutinoside) have also been identified by direct comparison of their respective molecular weights and retention time using mass spectrophotometer and high performance liquid chromatography (HPLC) (Kim et al., 2011). Akowuah et al. (2001, 2002) conducted preliminary phytochemical analysis on *Gynura procumbens* methanol extract, and this analysis led to isolation of flavonol and flavonol glycoside including rutin, quercetin, kaempferol, and quercetin-3-O-rhamnosyl (1-6) glucoside, quercetin-3-O-rhamnosyl (1-6) galactoside, kaempferol-3-O-rhamnosyl 1-6) glucoside and kaempferol-3-O-glucoside.

In addition, kaempferol-3-O-rutinoside and kaempferol-3-O-glucoside in the *Gynura procumbens* methanol extract both have been determined using high performance thin layer chromatography (Yam et al., 2009). Hassan et al. (2010) performed the same phytochemical analysis as Yam et al. (2009) towards *Gynura procumbens* aqueous extract and revealed that this plant extract contains 0.76% and 2.65% of kaempferol-3-O-rutinoside and kaempferol-3-O-glucoside respectively.

2.6 Drug Metabolism

Preclinical drug metabolism and pharmacokinetics is important in drug discovery and development (Gunaratna, 2000). Drug metabolism is defined as a process of elimination of foreign compound from the body. This process occurs mainly in the liver with the aid of enzymes. Enzymes which are responsible for converting or transforming or detoxifying foreign compound are called drug metabolizing enzymes. Drug metabolizing enzymes are separated into two groups including oxidative drug metabolizing enzymes and conjugative drug metabolizing enzymes. Oxidative drug metabolizing enzymes are also known as Phase I drug metabolizing enzymes includes cytochrome P450 (CYP450) and flavin monooxygenase (FMO), in which both catalyze the introduction of an oxygen atom into substrate molecules resulting in hydroxylation and demethylation. Phase I reaction involves the process of converting non-polar parent compound into a more polar or hydrophilic compound by the addition of functional groups such as –OH, -SH, -NH_2, -COOH.

The conjugative drug metabolizing enzymes or Phase II drug metabolizing enzymes include UDP-glyconosyltransferases (UGTs), glutathione transferases (GSTs), sulfotransferases (SULTs), and N-acetyltransferases (NATs). Phase II drug metabolism reaction involves the conjugation or the attachment of an ionized group to the substrate which then further increases aqueous solubility and decreases pharmacological activity of the substrate. This group comprises of glutathione, methyl group and acetyl group.

Phase I reaction occurs in liver microsome and usually precede Phase II reaction which takes place in the liver cells as the parent compound or the metabolite from Phase I becomes conjugated. Numerous factors are knowns to affect drug metabolism reaction including enzyme induction and enzyme inhibition. Enzyme induction results in acceleration of drug biotransformation and as a result loses its therapeutic effects due to rapid metabolism. In worst cases, certain drugs with active metabolites can exhibit increased adverse drug effects and/or toxicity. On the other hand, enzyme inhibition occurs when two drugs are metabolized via the same pathway and if one of the resulting drug products is a potent inhibitor, it can decrease the metabolism of the other drug thus leading to adverse toxicity (Ogu and Maxa, 2000). There are several factors that influence the activity of drug metabolism (Gibson and Skett, 1986) and these factors are summarized in Table 2.1 below.

Table 2.1: List of factors that affect the activity of drug metabolizing enzymes

Internal	External
Species	Diet
Genetic	Environment
Sex	
Age	
Hormonal control	
Disease	

*Adapted from Gibson and Skett, (1986)

To date, drug metabolism is one of the most intensely studied aspects in herb-drug interaction. Grapefruit juice has been found to disrupt the oral bioavailability of various CYP3A4 substrates including cyclosporin A, felodipine, midazolam, terfenadine, verapamil, saquinavir, and ethinyl estradiol (Schmiedlin-Ren et al., 1997).

In addition, Bhardwaj et al. (2002) reported that, piperine, a chemical constituent found in black pepper inhibited P-glycoprotein-mediated, polarized transport of digoxin and cyclosporin A transport in monolayers of Caco-2 cells (Bhardwaj et al., 2002). The same author also claimed that piperine inhibited CYP3A4-mediated formation of the verapamil metabolites D-617 and norverapamil (Bhardwaj et al., 2002). Since P-glycoprotein and CYP3A4 are important in determination of bioavailability of many drugs such as digoxin, verapamil and cyclosporin A, inhibition of both proteins by concomitantly administered substance such as piperine or grapefruit juice may lead to elevation of plasma concentration of a drug due to a dual effect on drug transport and metabolism (Schmiedlin-Ren et al., 1997; Bhardwaj et al., 2002).

2.7 Phase I Drug Metabolizing Enzymes

Cytochrome P450 or CYP450 isoenzymes are superfamilies of hemoproteins. The term P450 is derived from spectrophotometric absorption peak of the enzyme at a wavelength 450 nm when it is bounded and reduced by carbon monoxide (Chang and Kam, 1999). CYP450 isoenzymes are popular for their role in the metabolism of diverse exogenous materials such as drugs, environmental pollutants, and chemicals (Guengerich, 1999). Fifty seven CYP450 genes have been found in human and only CYP1, CYP2, and CYP3 families are known to mainly contribute to drug metabolism. Among CYP450 isoenzymes, only five accounts for major drug metabolism namely CYP3A4, CYP2D6, CYP2C9, CYP2C19, and CYP1A2 (Guengerich, 2003). CYP3A4 is the most important enzyme as it metabolizes almost all CYP450-mediated reactions (Guengerich, 1996).

Generally, xenobiotic compounds are removed from our body by oxidation reaction of CYP450 which then results in the formation of more water-soluble and less toxic metabolite. However, metabolic activation of carcinogens may also occur during the oxidative mechanism mediated by CYP enzymes (Gonzalez and Gelboin, 1994). For instance, CYP1 family is responsible for the metabolic activation of carcinogens such as benzo[α]pyrene and 7,12-dimethylben[α]anthracene (Slaga et al., 1979). Therefore, inhibitor of CYP1A2 may possess chemo preventive properties (Kim et al., 2013). Several studies have been conducted regarding herbal and natural constituents that have inhibitory effects on CYP1A family and these constituents may play as chemopreventive agents in carcinogenesis due to exposure to polycyclic aromatic hydrocarbon (PAH) (Hwang et al., 2008; Pekthong et al., 2008).

Pharmacological studies have reported kava extract and/or kavalactones, potent inhibitors of CYP3A4, may decrease elimination of alprazolam upon co-administeration of kava and alprazolam (Zhou et al., 2004). Other studies have reported that tanshinones, an active constituent in Danshen, (*Salvia miltiorrhiza*) inhibited various CYP probe substrates in both human liver microsomes and specific human isoforms *in vitro* (Wang et al., 2010). In addition, drug interaction has also been observed for the two known chemical constituents in Schisandra fruit extract (gomisin B and C) that showed a potent inhibitory effect on CYP3A4 activity comparable with that of ketoconazole, a known inhibitor for CYP3A4 enzyme (Iwata et al., 2004). Natural product has been consumed for different reasons. The leaves of *Mitragyna speciosa* or commonly known as ketum in Malaysia, have been used tradionally to treat various diseases.

However, recent studies revealed that alkaloid extract of *Mitragyna speciosa* is a potent inhibitor of CYP3A4 and CYP2D6 and moderate inhibitor of CYP1A2 (Kong et al., 2011).

2.8 CYP3A4 Isoform

The literature data on CYP3A4 inhibition by herbal medicines has increased over the years. Goldenseal (*Hydrastis canadensis*), St John's wort (*Hypericum perforatum*), cat's claw (*Uncaria tomentosa*), Black samson (*Echinacea angustifolia*), wild cherry (*Trifolium pratense*), chamomile (*Matricaria chamomilla*) and licorice (*Glycyrrhiza glabra*) significantly inhibited CYP3A4 metabolite with IC_{50} values lower than 1% to 2% of full strength (highest concentration) measured using fluorometric microtitre plate assay (Budzinski et al., 2000). Herbal components namely ginsenosides Rb1, Rb2, Rc, and Rd (from ginseng quercetin) and ginkgolides A and B (from Ginkgo biloba) were investigated for their inhibitory effect on CYP2C9 and CYP3A4 and all these herbal components showed different potencies in inhibiting both the CYPs (He and Edeki, 2004). Hyperforin, a putative active antidepressant constituent from St John's wort (*Hypericum perforatum*) extract was a potent competitive inhibitor of CYP3A4 activities with the inhibitor constant (K_i) value of 0.48μM (Obach, 2000). Ethanol extract of kava and three purified kava lactones including methysticin, desmethoxyyangonin, and yangonin were previously investigated for their inhibitory effects on CYP450 enzymes (CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4) expressed in a baculovirus / insect cell system and in cryopreserved human hepatocytes and it is found that they have IC_{50} values approximately 10μM (Zou et al., 2004).

By employing P450-Glo™ Screening System, Alpine lovage (*Mutellina purpurea* L.) methanol extract showed about 6 fold significant inhibition towards CYP3A4 compared to its aqueous extract (Sieniawska et al., 2012). In a cell-free system, schisandrol A (SCH) and gomisin A (GOM), two main dibenzocyclooctadiene lignants isolated from the fruit of *Schisandra chinensis*, inhibited CYP3A4 activity with IC_{50} value 32.02µM and 1.39µM respectively (Wan et al., 2010).

Four Malaysian medicinal plants namely *Andrographis paniculata*, *Orthosiphon staminues*, *Mitragyna speciosa* and *Curcuma xanthorrhiza* have been found to have the ability to inhibit CYP3A4 with IC_{50} value in the range from 28µg/mL to 300µg/mL (Hanapi et al., 2010). Earlier studies reported that, *Orthosiphon stamineus* dichloromethane and petroleum ether extract moderately inhibited CYP3A4 than aqueous and methanol, but eupatorin, the active constituent of *Orthosiphon staminues* was found potently inhibited CYP3A4 (Pan et al., 2011). In addition, the same author suggested that the inhibitory effect of dichloromethane and petroleum ether extract on CYP3A4 was probably because of the high content of eupatorin in dichloromethane and petroleum ether extracts and therefore, drug-herb interaction are likely to occur for CYP3A4 substrates (Pan et al., 2011). It is worth noting that, among 30 Indonesian medicinal plants, 4 medicinal plants (ethyl acetate soluble fraction), namely *Pi. cuceba*, *Pi. nigrum* fruit, *Pi nigrum* leaf, and *Z. aromaticum* showed inhibitory activity of more than 70% towards CYP3A4 (Usia et al., 2006). Interestingly, (-) -hinokinin, a compound that contain two methylenedioxyphenyl in its chemical structure, isolated from *Pi. Cuceba*, is a potent inhibitor towards CYP3A4 and therefore, the inhibitory effect of *Pi. Cuceba* on CYP3A4 may be due to this compound (Usia et al., 2006).

In contrast to Usia et al. (2006), schisandria fruit component which is schizandrin, a compound with no methylenedioxy group in its chemical structure, weakly inhibited CYP3A4 with an IC_{50} value more than 100µM (Iwata et al., 2004).

It is noteworthy that, perturbation in CYP450 enzyme activities may lead to treatment failure or worse, clinically fatal due to toxicity (Gomez-Lechon et al., 2008). Inhibition or induction of CYP450 enzymes may increase the drug plasma concentration level in the body. This situation may lead to toxicity or decrease the drug plasma concentration therefore, loses its therapeutic effect which may lead to treatment failure (Li et al., 1997). Based on the recent studies on *Elephantopus scaber L*. major constituent, a series of CYP induction and CYP inhibition have been done using P450 Glo™ CYP3A4 assay. Deoxyelephantopin (major germacranolide sesquiterpene lactone isolated from *E. Saber*) showed induction of CYP3A4 enzyme activity at a concentration range of 0.01 – 0.1µM. Oddly, when the concentration was further increased, CYP3A4 enzyme activity starts to decrease and thus, deoxyelephantopin was found to be a weak inducer and a weak inhibitor and unlikely to stimulate negative effect in human (Koe et al., 2013). It can be conclude that, if a drug/herb inhibits CYP3A4 that catalyzes the metabolism of a concomitant drug, plasma concentration of the concomitant drug will increase and thus, this will lead to toxicity. Table 2.2 shows the list of CYP3A4 substrates that may cause herb-drug interaction or drug-drug interaction when CYP3A4 inhibitors are taken concurrently with herbal medicines.

Table 2.2: List of CYP3A4 substrates

CYP3A4 substrates			
Alfentanil (Alfenta)	Carbamazepine (eg, Tegretol)	Erythromycin	Solifenacin (Vesicare)
Alfuzosin (Uroxatral)	Clonazepam (Klonopin)	Estazolam (ProSom)	Tinidazole (Tindamax)
Atorvastatin (Lipitor)	Cyclosporine (Neoral)	Ethosuximide (Zarontin)	Tipranavir (Aptivus)
Amlodipine (Norvasc)	Darunavir (Prezista)	Felodipine (Plendil)	Triazolam (Halcion)
Bexarotene (Targretin)	Dexamethasone (Decadron)	Flurazepam (Dalmane)	Verapamil (Calan)
Budesonide (Entocort)	Docetaxel (Taxotere)	Galantamine (Reminyl)	Vinblastine (Velbane)
Buprenorphine (Subutex)	Ergotamine (Ergomar)	Gefitinib (Iressa)	Vincristine (Oncovin)
Halofantrine (Halfan)	Indinavir (Crixivan)	Irinotecan (Camptosar)	Ziprasidone (Geodon)
Itraconazole (Sporanox)	Lopinavir (Kaletra)	Levomethadyl (Orlaam)	Zolpidem (Ambien)
Lovastatin (Mevacor)	Midazolam (Versed)	Nefazodone	Zonisamide (Zonegran)
Paclitaxel (Taxol)	Pioglitazone	Ranolazine (Ranexa)	Zopiclone (Imovane)
Repaglinide (Prandin)	Ritonavir (Norvir)	Simvastatin (Zocor)	Sildenafil (Viagra)

Adapted from Horn and Hansten (2008)

2.9 CYP1A2 Isoform

CYP1A2 is one of the members of CYP450 enzymatic group and its expression is induced by certain polycyclic aromatic hydrocarbons (PAHs). It has the ability to metabolize some PAHs into carcinogenic intermediates. The main CYP450 enzymes involved in carcinogen activation are CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2E1 and CP3A4. The most common enzymes from group CYP450 involved in cancer induction are both CYP1A2 and CYP2A6 (Guengerich, 2003). Several studies have been conducted exclusively regarding the issue that some herbal or natural constituents have the capability to inactivate CYP1A family members thus playing an important role in preventing the effects in carcinogenesis due to exposure to PAHs (Hwang et al., 2008; Pekthong et al., 2008). Standardized Asian ginseng (*Panax ginseng*) extract and standardized North American ginseng (*Panax quinquefolius*) extract inhibited human recombinant enzymes CYP1A1, CYP1A2, and CYP1B1 activities in a concentration-dependent manner (Chang et al., 2002). Mollugin, a pharmacological compound isolated from *Rubia cordifolia*, inhibited recombinant enzyme CYP1A2 competitively thus changing the pharmacokinetic properties of other drugs such as caffeine and theophylline inevitably (Kim et al., 2013). Tanshinones (tanshinones I, tanshinone IIA, and cryptotanshinone), major constituents of *Salvia miltiorrhiza,* competitively inhibited CYP1A2 with a K_i value of 1.5 – 2.5μM thus suggesting the occurrence of herb-drug interaction, given that CYP1A2 is solely responsible for the metabolism and disposition of almost all drugs currently used (Wang et al., 2010).

Therefore, in recent time, modulation of the activity of CYP1A2 by dietary phytochemicals such as flavonoids and elucidation of the structure of flavonoids that are responsible in herb-drug interaction have gain worldwide attention. Zhai et al. (1998) reported flavone and five hydroxylated derivatives of flavone showed different potencies and selectivities on inhibition of CYP1A enzymes. *Mitragyna speciosa* alkaloid extract showed moderate inhibition towards CYP1A2 with an IC_{50} value of 39μg/mL (Kong et al., 2011). According to Appiah-Opong et al. (2007), curcumin, a polyphenolic component of tumeric, inhibited CYP1A2 competitively with an IC_{50} value of 40.0µM. Kuo et al., (2004) stated that flavonols inhibited CYP1A enzyme in the decreasing order of aglycone >> monoglycoside > diglycoside. CYP1A2 metabolizes important medicines as listed in Table 2.3, therefore, suggesting that interaction between medicines and herbal extract that metabolize by the same enzyme may increase the plasma concentration of those particular medicines that if it is taken concurrently.