ANALYSIS OF PHYTOCHEMICALS, ANTIOXIDANT AND ANTIHYPERLIPIDEMIC ACTIVITIES OF METHANOLIC AND AQUEOUS EXTRACTS OF VARIOUS PARTS OF *GARCINIA MANGOSTANA*

by

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LIST OF SYMBOLS AND ABBREVATIONS

%	Percentage
°C	Degree Celsius
α	Alpha
β	Beta
γ	Gamma
μ	Micro
AAPH	2,2'-Azobis(2-amidinopropane) dihydrochloride
ABTS	2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid)
ACAT	Acyltransferase
AEC	Animal Ethics Committee
AH	Antioxidant
A.I	Atherogenic index
$AlCl_3$	Aluminum chloride
ALT	Alanine
ANOVA	Analysis of variance
ArOH	Aromatic ring
AST	Aspartate aminotransferases
B.subtilis	*Bacillus subtilis*
CAT	Catalase
CE	Cholesteryl ester
CETP	Chlolesteryl ester transfer protein
CMC	Carboxymethyl cellulose
COX-2	Cyclooxygenase 2

cm	Centimeter
CVD	Cardiovascular diseases
DMBA	7,12-dimethylbenz[a]anthracene
DMH	1,2-dimethylhydrazine
DPPH	2, 2-diphenyl-1-picrylhydrazyl
EC_{50}	Half maximal effective concentration
ET	Electron transfer
FCR	Folin-Ciocalteu reagent
Fe (II)-TPTZ	Ferrous tripyridyltriazine
Fe (III)-TPTZ	Ferric tripyridyltriazine
$Fe(SO_4)_2$	Ferric sulphate
$FeCl_2$	Iron (II) chloride
$FeCl_3$	Ferric chloride
FRAP	Ferric reducing antioxidant power
g	Gram
g/kg	Grams per kilogram
G. mangostana	*Garcinia mangostana*
H	Hydrogen
$H^{\cdot}$	Hydrogen radical
H_2O_2	Hydrogen peroxide
HAT	Hydrogen atom transfer
HDL-C	High density lipoprotein cholesterol
HC	Hyperlipidemic control
HL	Hepatic lipase
HMG-CoA	3-hydroxy-3-methylglutaryl- CoA

HPTLC	High Performance Thin Layer Chromatography
hr	Hour
hrs	Hours
IDL-C	Intermediate density lipoproteins cholesterol
i.p	Intraperitonial injection
kg	Kilogram
LCAT	Lecithin cholesterol acetyltransferase
LDL-C	Low density lipoprotein cholesterol
LPL	Lipoprotein lipase
LPS	Lipopolysaccharide
M	Molar
mg/mL	Milligrams per liter
min	Minutes
mL	Milliliter
mM	Millimolar
mmol/L	Millimoles per liter
MRSA	Methicillin or multi-resistant *Staphylcoccus aures*
Na_2CO_3	Sodium carbonate
$NaNO_2$	Sodium nitrate
NaOH	Sodium Hydroxide
NC	Normal control
NCEP	National Cholesterol Education Program Adult Treatment Panel
NO	Nitric oxide
nm	Nanometer
$OH^{\cdot}$	Hydroxyl radical

$O^{\cdot}_2^-$	Superoxide anion
$OONO^-$	Peroxynitrite
P-407	Poloxamer 407
P.aeruginosa	*Pseudomonas aeruginosa*
PG	Prostaglandins
pH	Power of hydrogen
r^2	Regression value
R_f	Retention value
PML	Polymorphonuclear leukocytes
$ROO^{\cdot}$	Peroxyl radical
ROOH	Hydroperoxides
ROS	Reactive oxygen species
S.aureus	*Staphylococcus aureus*
SD	Standard deviation
SEM	Standard error of mean
SET	Single electron transfer
SOD	Superoxide dismutase
spp.	Species
S.typhimurium	*Salmonella typhimurium*
TC	Total cholesterol
TFC	Total flavonoid content
TG	Triglycerides
TPC	Total phenolic content
TPTZ	2,4,6-tripyridyl-s-triazine
µg	Microgram

µg/g	Microgram per gram
µg CE/mg	Microgram catechin acid equivalent per microgram
µg GAE/mg	Microgram gallic acid equivalent per microgram
µg/mL	Microgram per milliliter
µL	Microliter
USA	United States of America
VLDL-C	Very low density lipoprotein cholesterol
v/v	Volume over volume
WHO	World Health Organization

ANALISIS FITOKIMIA, AKTIVITI ANTIOKSIDAN DAN AKTIVITI ANTIHIPERLIPIDEMIK DARI EKSTRAK METANOL DAN AKUES PELBAGAI BAHAGIAN *GARCINIA MANGOSTANA*

ABSTRAK

Garcinia mangostana adalah tumbuhan tropika yang biasanya terdapat di Asia Tenggara. Tumbuhan ini mempunyai kesan-kesan antiradang, antibakteria, antiulser, antiseptik dan potensi untuk mencegah kerosakan pengoksidaan kolesterol lipoprotein berketumpatan rendah (LDL-C). Kajian ini bertujuan untuk menilai aktiviti antioksidan dan aktiviti antihiperlipidemik ekstrak metanol dan akueus pelbagai bahagian *G. mangostana*, iaitu daun, batang, kulit kayu, kulit buah, kelopak, tangkai, benih dan isi buah. Jumlah kandungan fenol (TPC) tertinggi adalah ekstrak methanol daun (41.74 μg GAE/g) dan ekstrak akueus daun (11.29 μg GAE/g). Ekstrak daun juga nenunjukkan jumlah kandungan flavonoid (TFC) teringgi dengan 31.80 μg GAE/g bagi ekstrak methanol dan 19.48 μg GAE/g bagi ekstrak akueus daun. Bagi kuasa antioksida penurunan ferik (FRAP), kedua-dua ekstrak metanol dan akueus menunjukkan aktiviti FRAP tertinggi dengan 0.45 ± 0.01 μmol Fe^{2+}/g dan 0.39 ± 0.09 μmol Fe^{2+}/g masing-masing, manakal α-mangostin mempunyai aktiviti FRAP yang rendah berbanding dengan ekstrak. Ekstrak metanol daripada tangkai menunjukkan aktiviti menghapus radikal 2-difenil-1-pikrihidrazil (DPPH) tertinggi dengan IC_{50} 3.36 ± 0.52 μg/ mL, sementara ekstrak akueus daun menunjukkan aktiviti menghapus DPPH tertinggi dengan IC_{50} 3.21 ± 0.57 μg/mL berbanding α-mangostin yang mempunyai IC_{50} 4.03 ± 0.87 μg/mL. Bagi aktiviti menghapus radikal 2,2-azinobis (3-etil- benzotiazolina-6-asid sulfer) (ABTS), ekstrak metanol batang dan ekstrak akueus daun menunjukkan aktiviti tertinggi dengan nilai IC_{50} 0.78 ± 0.45

μg/mL dan IC_{50} 0.81 ± 0.37 μg/mL, masing-masing dan α-mangostin mempunyai IC_{50} 2.88 ± 0.55 μg/mL. Aktiviti antihiperlipidemik bagi ekstrak metanol dan akueus daripada daun, batang dan kulit buah *G. mangostana* dalam tikus hiperlipidemik teraruh P-407, mendapati bahawa ekstrak akueus menunjukkan pengurangan lebih tinggi bagi paras jumlah kolesterol (TC), trigliserida (TG), LDL-C dan peningkatan kolesterol lipoprotein berketumpatan tinggi (HDL-C) berbanding dengan ekstrak metanol. Ekstrak akueus daun menunjukkan pengurangan yang signifikan iaitu 62.95 % dan 82.08 % bagi nilai TC ($p<0.001$) dan TG ($p<0.001$), masing-masing pada 58 jam selepas induksi hiperlipidemia. Walau bagaimanapun, α- mangostin pada dos 50 mg/kg dan 100 mg/kg tidak menunjukkan pengurangan bagi TC dan TG pada 58 jam selepas induksi hyperlipidemia. Penilaian lanjut aktiviti antihiperlipidemik bagi ekstrak akueus daun *G. mangostana* dalam tikus hiperlipidemik teraruh diet berkolesterol tinggi menunjukkan ekstrak daun pada 1000 mg/kg menunjukkan pengurangan tertinggi bagi TC, TG, LDL-C dan peningkatan bagi HDL-C berbanding dengan 250 mg/kg dan 500 mg/kg ekstrak. Pemberian ekstrak daun1000 mg/kg menunjukkan pengurangan yang signifikan iaitu, 90.51 % dan 92.68 % bagi nilai TC ($p<0.001$) dan TG ($p<0.001$), masing-masing berbanding dengan kumpulan kawalan hiperlipidemik. Keputusan ini menunjukkan bahawa pengurangan TC dan TG dalam tikus hiperlipidemik teraruh diet berkolesterol tinggi bergantungan dos. Kuantifikasi α-mangostin dalam ekstrak metanol dan akues pelbagai bahagian dengan menggunakan kaedah HPTLC menunjukkan bahawa jumlah kandungan tertinggi ditemui dalam ekstrak metanol dari kulit buah (12.54 %) dan ekstrak akueus kulit kayu (2.2 3 %). α-mangostin didapati bukan sebatian bioaktif yang bertanggungjawab bagi aktiviti antioksidan dan antihiperlipidemik ekstrak. Sebagai kesimpulan kajian ini mendapati bahawa ekstrak akueus daun *G.mangostana*

berpotensi sebagai agen farmakologi bagi mencegah kerosakan pengoksidaan LDL-C dan merawat hiperlipidemia. Bagaimanapun, pemencilan lanjut dan pencirian sebatian bioaktif ekstrak yang bertanggungjawab untuk penurunan lipid adalah diperlukan.

ANALYSIS OF PHYTOCHEMICALS, ANTIOXIDANT AND ANTIHYPERLIPIDEMIC ACTIVITIES OF METHANOLIC AND AQUEOUS EXTRACTS OF VARIOUS PARTS OF *GARCINIA MANGOSTANA*

ABSTRACT

Garcinia mangostana is a tropical plant found commonly in South East Asia. This plant possesses anti-inflammatory, antibacterial, antiulcer, antiseptic effects and showed prevention against oxidative damage of low-density lipoprotein cholesterol (LDL-C). The present study aimed to evaluate the antioxidant and antihyperlipidemic activities as well as phytochemical analysis of methanolic and aqueous extracts of various parts of leaves of *G. mangostana*, namely leaves, stem, bark, hull, petals, stalk, seed and flesh. The highest phenolic content was found in methanolic (41.74 μg GAE/g) and aqueous (11.29 μg GAE/g) extracts of leaves. Leaves extracts also showed highest flavonoid content of 31.80 μg GAE/g in methanolic and 19.48 μg GAE/g in aqueous extracts. For ferric reducing antioxidant power (FRAP), both methanolic and aqueous extracts of leaves showed most potent activity of 0.45 ± 0.01 μmol Fe^{2+}/g and 0.39 ± 0.09 μmol Fe^{2+}/g respectively while α-mangostin possessed low FRAP activity compared to the extracts. Methanolic extract of stalk showed highest 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity with EC_{50} of 3.36 ± 0.52 μg/mL while aqueous extract of leaves showed EC_{50} of 3.21 ± 0.57 μg/mL compared to α- mangostin which had EC_{50} of 4.03 ± 0.87 μg/mL. For 2,2-azinobis (3-ethyl- benzothiazolin-6-sulfate acid) (ABTS) radical scavenging activity, methanolic extract of stem and aqueous extract of leaves showed highest activity with EC_{50} of 0.78 ± 0.45 μg/mL and 0.81 ± 0.37 μg/mL, respectively plus α-mangostin had EC_{50} of 2.88 ± 0.55 μg/mL. The antihyperlipidemic activity of the

methanolic and aqueous extracts of leaves, stem and hull of *G. mangostana* in P-407-induced acute hyperlipidemic rats demonstrated that the aqueous extracts exhibited larger reduction in total cholesterol (TC), triglycerides (TG), and LDL-C and significant increase in high density lipoprotein cholesterol (HDL-C) levels compared to methanolic extracts. Aqueous extract of leaves showed significant reduction of 62.95 % and 82.08 % in TC ($p<0.001$) and TG ($p<0.001$) levels, respectively at 58 hrs after hyperlipidemia induction. However, α-mangostin at doses of 50 mg/kg and 100 mg/kg showed no significant reduction of TC and TG levels at 58 hrs after hyperlipidemia induction. Further antihyperlipidemic evaluation of aqueous extracts of leaves in high fat diet-induced chronic hyperlipidemic rats showed that the leaves extract at 1000 mg/kg exhibited larger reduction in TC, TG, LDL-C and increase in HDL-C levels compared to 250 mg/kg and 500 mg/kg of extracts. The administration of 1000 mg/kg of leaves extract showed significant reduction of 90.51 % and 92.68 % in TC ($p<0.001$) and TG ($p<0.001$) levels, respectively compared to hyperlipidemic control after 4 weeks of treatment. This result indicates that the aqueous extract of leaves exhibited a dose-dependent reduction in reducing serum TC and TG levels. Quantification of α-mangostin in various parts of methanolic and aqueous extracts using high performance thin layer chromatography (HPTLC) analysis showed that high amount of α-mangostin was found in methanolic extract of hull (12.54 %) and aqueous extract of bark (2.23 %). α-mangostin was found to be not the main bioactive compound that responsible for the antioxidant and antihyperlipidemic activities of the extracts. In conclusion, the present study found that aqueous extract of *G. mangostana* leaves could be potential pharmacological agents in preventing oxidative damage of LDL-C and treating hyperlipidemia.

However, further isolation and characterization of bioactive compounds that are responsible for the lipid lowering effect is warranted.

CHAPTER 1

INTRODUCTION

Hyperlipidemia is defined as an abnormal elevation of total cholesterol, low-density lipoprotein cholesterol (LDL-C) and/ or triglycerides (TG) levels in the blood (Mishra et al., 2011; Sudha et al., 2011). Hyperlipidemia is known to be one of the prime risk factor for ischemic cardiovascular diseases such as atherosclerosis (Amran et al., 2009). Globally, a third of ischemic heart disease is attributable to high cholesterol (World Health Organization, 2014). Overall, raised cholesterol is estimated to cause 2.6 million deaths (4.5 % of total) and 29.7 million disability adjusted life years (DALYS), or 2.0 % of total DALYS (World Health Organization, 2014). The World Health Organization (WHO) has also predicted that heart diseases and stroke are becoming more deadly, with a projected combined death toll of 24 million by 2030 (Reinhardt, 2005).

Therefore, to manage hyperlipidemic several groups of drugs have been developed such as statins, fibrates and niacin commonly known as lipid-lowering drugs. Statins reduce cholesterol by interfering with the cholesterol biosynthetic pathway; fibrates decrease fatty acid and TG levels, while niacin reduce circulating TG and LDL-C and increase high density lipoprotein cholesterol (HDL-C) (Osumi et al., 1985; Krukemyer et al., 1987; Hebert et al., 1997; Trevor et al., 1999; Vasudevan and Jones, 2005; Pahan, 2006). Statins display a broad spectrum of activities in addition to their lipid-lowering properties but certain statins were shown to be associated with liver

function abnormalities and occasionally, rhabdomyolysis (Miller et al, 2001; Pahan, 2006; Sook et al., 2011).

There is a growing interest to discover herbal medicine for management of hyperlipidemia. Plants have formed the basis of traditional systems of medicine that have been in existence for thousands of years to treat human ailments and diseases as well to provide mankind with new remedies (Gurib-Fakim, 2006). Traditional medicine involves the use of medicinal plants as the integral component of the medicinal systems (Saikat, 2011). Medicinal plants still remain significant as natural alternatives to synthetic drugs with about 80 % of the world population depending upon plants for their primary health care according to WHO estimation (Payyappallimana, 2002). World ethno botanical information reported that a number of herbal medicines from plants and vegetables are used for controlling hyperlipidemia (Mishra et al., 2011). Recent study has shown that medicinal plants intake in rats results in an increase of antioxidant enzymes activity plus HDL-C level, and a decrease in malondialdehyde, which may reduce the risk of heart disease (Eun and Jae, 2005).

Among the promising medicinal plants, that gain interest in controlling hyperlipidemia is *Garcinia mangostana,* which is a tropical plant found commonly in South East Asia (Apinya et al., 2009). This plant possesses a great variety of pharmacological activities including anti-inflammatory, antibacterial, antiulcer, antiseptic and prevention against oxidative damage of LDL-C (Gopalakrishnan and Shankaranarayanan, 1980; Williams et al., 1995; Nakatani et al., 2002; Chomnawang et al., 2005). The fruits of *G. mangostana,* which was extracted with different solvents

such as water, methanol and ethanol, inhibit the rise in plasma lipids and increase in HDL-C in rats fed with cholesterol containing diets (Leontowicz et al., 2006).

The bioactive secondary metabolites of *G. mangostana* are mainly the xanthone derivatives (Peres et al., 2000; Jung et al., 2006) and the α-mangostin being the major constituent of *G. mangostana* (Obolskiy et al., 2009). α-mangostin acts as a free radical scavenger and was found to prevent oxidation of LDL-C, thus being a potent substance for preventing the development of atherosclerosis (Williams et al., 1995; Obolskiy et al., 2009)

To date, there is no thorough study on antihyperlipidemic activity of *G. mangostana.* The majority of investigations on *G. mangostana* were focused on extraction and structure elucidation of xanthones from the fruit hull or pericarp of the fruit (Asai et al., 1995; Mahabusarakam et al., 1987; Suksamrarn et al., 2002; Puripattanavong et al., 2006; Obolskiy et al., 2009). Thus, in the present study, detailed work was undertaken to evaluate the antioxidant and antihyperlipidemic activity of the methanolic and aqueous extracts of various parts of *G. mangostana* in poloxamer 407-induced acute hyperlipidemic and high fat diet-induced chronic hyperlipidemic rat model.

1.1 Problem statements

According to WHO, high cholesterol is known as prime risk factor for coronary cardiovascular diseases such as atherosclerosis which is estimated to cause 4.5 % of deaths globally (World Health Organization, 2014). A study on the burden of disease

using DALYS showed that the major leading disease in Malaysia is coronary heart disease, which is attributable to high cholesterol. The total number of deaths in Malaysia resulted from coronary heart disease is at about 32% of the total deaths in the country (World Health Organization, 2011). Therefore, to manage hyperlipidemic several groups of drugs have been developed commonly known as lipid-lowering drugs. However, the growing interest of discovering herbal medicine for management of hyperlipidemia gains interest in investigating the potential of *G. mangostana* since preliminary screening among some local medicinal plants have indicated that extract of fruit hull has potential antihyperlipidemic activity. The antioxidant activity of the fruit and hull that responsible in preventing oxidative damage of LDL-C has also been reported previously. To date there is no thorough report on the comparative antihyperlipidemic or antioxidant activities of other parts of *G. mangostana*. Furthermore, it is not known whether the two activities were mainly due to the major constituent of *G. mangostana*, α-mangostin. Therefore, the present study was undertaken to address these questions scientifically.

1.2 Objectives of present study

The objectives of the present study are as following:

- to determine antioxidant potential of methanolic and aqueous extracts of various parts of *G. mangostana*.
- to evaluate antihyperlipidemic effect of methanolic and aqueous extracts of various parts of *G. mangostana* and its major constituent, α-mangostin in poloxamer 407-induced acute hyperlipidemic rats model.

- to evaluate antihyperlipidemic effect of most active extract of *G. mangostana* (selected from acute hyperlipidemia study) in high fat diet- induced chronic hyperlipidemic rats model.

- to quantify the major constituent, α-mangostin in methanolic and aqueous extracts of various parts of *G. mangostana* using high performance thin layer chromatography (HPTLC) method and correlate with their antioxidant and hyperlipidemic activities.

CHAPTER 2

LITERATURE REVIEW

2.1 Hyperlipidemia

Cholesterol is a wax-like steroid molecule, which serves as essential cell membrane component, substrate for the synthesis of steroid hormones including estrogen and androgen, precursor to vitamin D and precursor for the synthesis of bile acids, which emulsify dietary fats for absorption (Chen et al., 2008). Triglycerides (TG) are storage lipids that have a vital role in metabolism and energy production where it provides twice more energy than carbohydrates and protein. Lipids are not capable of move independently in the bloodstream. Therefore, lipids are transported as complexes of lipid and protein known as lipoproteins (Rang et al., 2012).

There are four main classes of lipoprotein where each differ in size and density; high density lipoprotein cholesterol (HDL-C), which build up of density > 1.063 g/mL and 5–15 nm in diameter, low density lipoprotein cholesterol (LDL-C) is between 1.019–1.063 g/mL (18–28 nm), very low density lipoprotein cholesterol (VLDL-C) is between 0.95–1.006 g/mL (30–80 nm), and chylomicron < 0.95 g/mL (100-1000 nm) (Garrett and Grisham, 1995). Each class of lipoprotein has a specific role in lipid transport. Chylomicron transports dietary cholesterol and TG from intestine to adipose tissues and skeletal muscles, VLDL- C transports newly synthesized TG and cholesterol from liver to adipose tissues and skeletal muscles, LDL-C provides cholesterol to those tissues that need it and HDL-C removes excessive cholesterol from peripheral tissues back to the liver and maintain cholesterol homeostasis in blood (Chen et al., 2008). LDL-C is considered as "bad" cholesterol and HDL-C known as "good" cholesterol. Hyperlipidemia is

characterized by raised of total cholesterol (TC), TG, LDL-C, VLDL-C as well as reduced HDL-C levels, which lead to lipid disorders such as atherosclerosis and cardiovascular diseases (Jeyabalan and Palayan, 2008). Hyperlipidemia is classified into six types according to the Fredrickson classification based on which lipoprotein type is increased (Mahamuni et al., 2012). Type I hyperlipidemia is characterized by elevation of TC, TG and excess of chylomicrons in the plasma due to lipoprotein lipase (LPL) and apo C-II deficiency. Type IIa hyperlipidemia is characterized by elevated or normal level of TC and elevation of LDL-C where familial hypercholesterolemia as the primary cause and hypothyroidism as the secondary cause. Type IIb hyperlipidemia includes elevation of TC, TG, LDL-C and VLDL-C levels.

Type III hyperlipidemia is characterized by elevation of TC, TG and excess chylomicron remnants and intermediate density lipoprotein cholesterol (IDL-C). The primary cause is familial type III hyperlipoproteinemia whereas hypothyroidis diabetes and obesity are the secondary causes. Elevated or normal level of TC, elevation of TG and excess of VLDL-C is classified as type IV hyperlipidemia. Familial combined hyperlipidemia, familial hypertriglyceridemia are the primary causes, and diabetes plus chronic renal diseases are the secondary causes of type IV hyperlipidemia. Lastly, type V hyperlipidemia is characterized by elevation of TC and TG plus excess of chylomicron and VLDL-C. The primary causes are familial hypertriglyceridemia and apoC-II deficiency while alcohol, diuretics, β blockers and diet are the secondary causes (Mahamuni et al., 2012). The classification of the hyperlipidemia is summarized in table 2.1.

Table 2.1: Fredrickson/ World Health Organization classification of hyperlipidemia (Mahamuni et al., 2012)

Type	Lipoprotein elevated	Cholesterol	Triglycerides	Atherosclerosis risk
I	Chylomicron	+	+	NE
IIa	LDL-C			High
IIb	LDL-C + VLDL-C	+	+	High
III	IDL-C	+	+	Moderate
IV	VLDL-C		+	Moderate
V	Chylomicron + VLDL-C	+	+	NE

+ indicates increased concentration; NE indicates not elevated.

2.1.1 Biosynthesis pathway of cholesterol

Dietary food and liver biosynthesis are the two sources of cholesterol for our body. Cholesterol is synthesized in liver and circulates through blood to most tissues whereas the dietary cholesterol is absorbed in the gut and transported to the liver (Liscum, 2002). Liver absorbs more cholesterol into the body during the intake of saturated and trans fats because cholesterol is soluble in fats, and dissolved cholesterol are easier to absorb into body (Go et al., 2014). Figure 2.1 shows the biosynthetic pathway of cholesterol.

In cholesterol biosynthesis, the soluble enzyme acetoacetyl-CoA thiolase interconvert acetyl-CoA and acetoacetyl-CoA, which is then condensed by 3-hydroxy-3-methylglutaryl (HMG-CoA). Further reactions produce mevalonate, which will be metabolized by a series of enzymes to squalene. Finally, squalene is then converted to cholesterol by a series of oxidation, reduction and demethylations (Liscum, 2002).

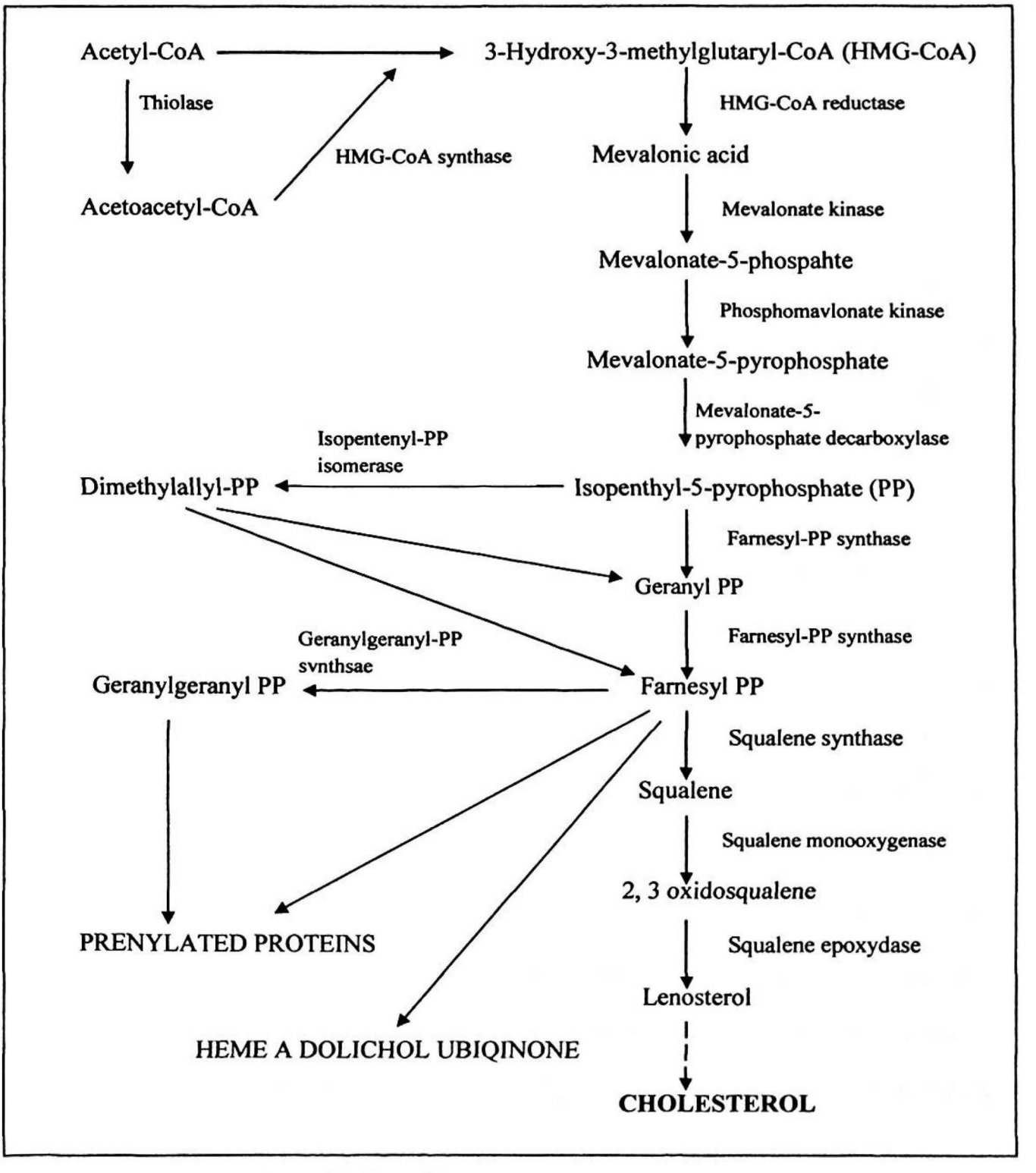

Figure 2.1: Biosynthesis pathway of cholesterol (Vance et al., 2002)

The processing of cholesterol is subdivided into exogenous and endogenous pathway. In the endogenous pathway, the liver and extra hepatic tissues synthesize cholesterol, which is then secreted into bloodstream, while intestine is the primary site of the exogenous pathway for dietary cholesterol uptake (Russell, 1992; Beisiegel, 1998). Endogenous cholesterol is secreted into bile or incorporated as free or esterified form into VLDL-C and LDL-C, which is then secreted into blood. Raised level of cholesterol in liver leads to an increased production of VLDL-C and LDL-C, along with down-regulation of the LDL-C receptor. Increase in the lipoprotein production and decrease in LDL-C clearance both lead to elevation of serum cholesterol level (Shepherd, 2001). Conversely, exogenous cholesterol is derived from bile and dietary sources, such as animal and dairy food products, which results in transfer of cholesterol to liver. Subsequent to absorption, the free cholesterol is re-esterified to cholesteryl ester (CE) by cholesterol acyltransferase (ACAT), and coupled with other lipids into chylomicrons, which are then secreted into the mesenteric lymph and finally into bloodstream (Dawson and Rudel, 1999). Once enter the bloodstream, chylomicrons are hydrolyzed by LPL at the endothelial surface of vessels and remnants chylomicron particles are formed. These chylomicron remnants then removed from the circulation into the liver whereas, tiny remnants may able to penetrate the endothelial surface of the arterial wall, where they may contribute to plaque formation (Mamo et al., 1998). Figure 2.2 shows the endogenous and exogenous pathways of cholesterol metabolism.

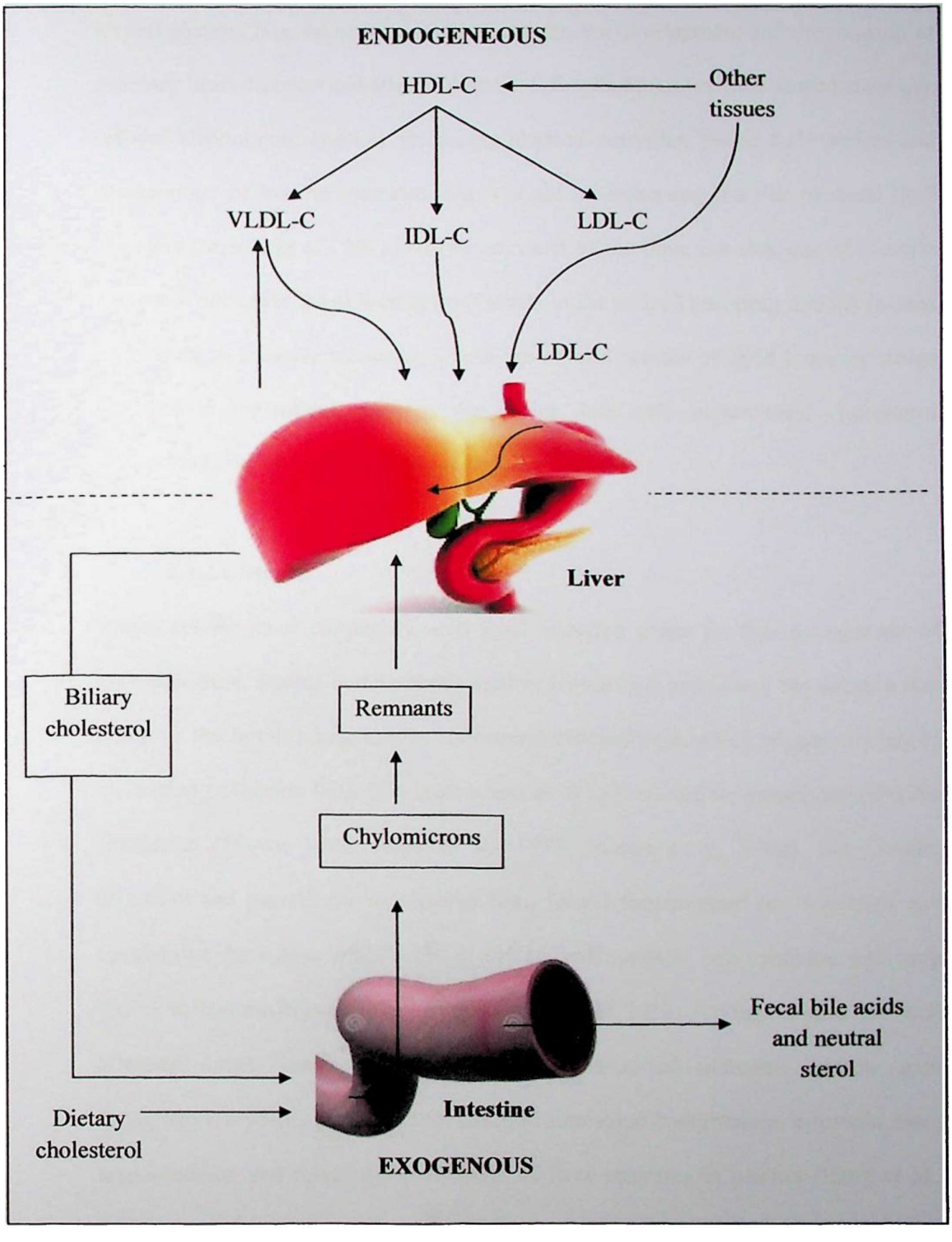

Figure 2.2: Endogenous and exogenous pathways of cholesterol production and clearance (Shepherd. 2001)

2.1.2 Treatment of hyperlipidemia

Hyperlipidemia is as an important risk factor for the development and progression of coronary heart diseases and atherosclerosis. Lifestyle measures such as quit smoking, reduced alcohol consumption, increasing physical activities, losing body weight and consumption of low fat saturated diet will aid in decreasing the risk of these lipid disorders (Mishra et al., 2011). However, most of the time, the changes of lifestyle alone will not lower the abnormal lipid levels in the body. Thus, drug therapy is used in addition to lifestyle measures. There are several classes of lipid lowering drugs available in the market such as the statins, bile acid sequestrants, cholesterol absorption inhibitors, fibrates and niacin.

2.1.2.a Statins

Statins are the most commonly used lipid lowering drugs for the management of hyperlipidemia. Statins competitively inhibit HMG-CoA reductase, the enzyme that catalyzes the rate-limiting step in cholesterol biosynthesis, which triggers increased expression of hepatic LDL-C receptors and clear LDL-C and its precursors from the circulation (Brown 1986; Endo et al., 1977; Maron et al, 2000). Simvastatin, lovastatin and pravastatin are derived from fungal fermentation are reversible and competitive inhibitors while atorvastatin and rosuvastatin are synthetic and long lasting inhibitors (Maron et al., 2000; Rang et al., 2012). Though statins are well tolerated drugs, some adverse affects were reported including muscle pain (myopathy), myositis (rhabdomyolysis) gastrointestinal disturbances, insomnia, rash, angio-oedema and raised concentrations of liver enzymes in plasma (Rang et al., 2012).

2.1.2.b Bile acid sequestrants

Bile acids are the major metabolite of cholesterol breakdown and bile acid absorption inhibitors are known as bile acid sequestrants (Chen et al., 2008). Cholestyramine and colestipol are the two bile acid sequestrants available for clinical use. According to National Cholesterol Education Program Adult Treatment Panel (NCEP), bile acid sequestrants are defined as drugs of first choice for lowering LDL-C efficiently (Davidson et al., 1999). The bile acid sequestrants interrupt the enterohepatic circulation of bile acids by binding with them in the intestine and form an insoluble complex that is excreted in feces. The increased excretion leads to an increase in bile acids synthesis from cholesterol in the liver. This increases the expression of LDL-C receptors, which remove cholesterol from the circulation and decrease the LDL-C level in bloodstream (Chen et al., 2008). However, the sequestrants can cause some gastrointestinal effects including gas, bloating, abdominal pain, nausea, vomiting and constipation (Davidson et al., 1999).

2.1.2.c Cholesterol absorption inhibitors

Ezetimibe is another class of lipid lowering drug that inhibits the intestinal absorption of dietary cholesterol by blocking passage across the intestinal wall without affecting absorption of TG, fat-soluble vitamins and other dietary nutrients (Gagne et al., 2002). Ezetimibe produced significant decreases in LDL-C and significant increases in HDL-C when administered to patients with primary hypercholesterolemia (Knopp et al., 1999; Dujovne et al., 1991). Ezetimibe is well-tolerated drug but may cause abdominal pain, headache, diarrhea, rash and angio-oedema (Rang et al., 2012).

2.1.2.d Fibrates

Fibrates are one of the widely used lipid-lowering drugs, which reduce serum TG, VLDL-C along with a moderate decrease in LDL-C and an increase in HDL-C (Staels et al., 1998). There are a number of fibrates available including bezafibrate, ciprofibrate, gemfibrozil, fenofibrate, and clofibrate (Rang et al., 2012). Five mechanisms have been suggested to explain the lipid lowering activity of fibrates: (a) induction of lipoprotein lipolysis, (b) induction of hepatic fatty acid (FA) uptake and reduction of hepatic TG production, (c) increased removal of LDL-C particles. (d) reduction in neutral lipid (cholesteryl ester and TG), exchange between VLD-C and HDL-C and (e) increase in HDL-C production and stimulation of reverse cholesterol transport with increase in the production of apoA-I and apoA-II in liver (Bart et al., 1998). Rhabdomyolysis, acute renal failure and predisposition of gallstone are the adverse side effects of the fibrates (Rang et al., 2012).

2.1.2.e 1 Nicotinic acid

Nicotinic acid or also known niacin is a vitamin, essential for many metabolic processes. It is used in gram quantities as lipid lowering agent where it is converted to nicotinamide that inhibits hepatic VLDL-C secretion and reduce circulating TG and LDL-C as well as increase HDL-C. Nevertheless, nicotinic acid may cause some unwanted side effects such as flushing, palpitations, impair glucose tolerance, gout and gastrointestinal disturbance (Rang et al., 2012).

There are two options available to regulate serum lipid levels in body including, block its uptake into the body with lipid- lowering drugs such as cholesterol absorption inhibitors, or promote its removal from the circulation by blocking the endogenous pathway with agents such as statins (Shepherd, 2001). Hence, combination therapy of more than one lipid-lowering drug would have an additive effect on plasma cholesterol levels. Combining a bile acid sequestrants with either statins or nicotinic acid have been shown to induce regression of atheroscelosis while stains and ezetimibe have an enhanced effect on removal of LDL-C from the circulation (Benson and Hickey, 1994; Shepherd, 2001). The combination drug therapy leads to a synergistic effect on lipids because these drugs work via different mechanisms (Benson and Hickey, 1994).

2.2 Medicinal plants

For thousands of years plants have been the basis of traditional medicine and continue to provide humankind with numerous new remedies. There are several plant-based traditional medicine systems such as ayurveda, unani, traditional Chinese medicine (TCM) and traditional Malay medicine, which has been used for ages in many countries (Gurib-Fakim, 2006). Throughout the ages, most of the plants have been used as medicines in the form of crude drugs such as tinctures, teas, poultices, powders and other herbal formulations (Balick and Cox, 1997; Samuelsson, 2004). According to World Health Organization (WHO), approximately 80 % of world's population still depends on traditional medicines for their primary health care needs (Farnsworth et al., 1985).

Traditional medicine has provided many important modern drugs such as morphine, cocaine, codeine, digitoxin and quinine whereby some are still in use

(Newman et al., 2000; Butler, 2004; Samuelsson, 2004). The drug discovery from medicinal plants has involved isolation of active compounds, starting with the isolation of morphine from opium in the early 19th century (Kinghorn, 2001; Samuelsson, 2004). There are also many new medicinal plant-derived drugs that have been recently discovered. For instance, galantamine isolated from *Galanthus woronowii* in Russia (Heinrich and Teoh, 2004; Pirttila et al., 2004). Arteether is an antimalarial drug that derived from artemisinin, a sesquiterpene lactone isolated from *Artemisia annua*, a plant used in traditional Chinese medicine (TCM) (Agtmael et al., 1999; Graul, 2001). Many more drugs derived from medicinal plants have been broad pharmacological effects such as such as anticancer, antibacterial, antifungal, antidiabetic.

Mixtures of different chemical compounds in the medicinal plants may act individually, additively or in synergy to improve health. For example, one medicinal plant may possibly have bitter substances that stimulate digestion, phenolic compounds that serve as antioxidant, anti-inflammatory compounds that lessen swellings and pain, and alkaloids that improve mood and provide a sense of well-being. Furthermore, traditional and allopathic medicine occurs side by side in a complimentary way (Gurib-Fakim, 2006).

2.2.1 Herbal medicines with antihyperlipidemic properties

Several herbal medicines have been used in the treatment of hyperlipidemia such as red yeast rice (*Monascus purpureus*), garlic (*Allium sativum*), black tea and green tea, which caused reduction in TC and LDL-C concentrations. Red yeast rice contains naturally occurring lovastatin (monacolin K) and other monacolins that may inhibit HMG-C◠A reductase and reduce LDL-C levels. Monounsaturated fatty acids

in red yeast rice aid in lowering cholesterol levels. TC and LDL-C levels dropped to baseline in 8 weeks in 83 hyperlipidemia patients while HDL-C level was unaffected (Heber et al., 1999).

Green tea may have a greater inhibitory effect on the intestinal absorption of lipids due to its higher catechins levels (Ikeda et al., 1992). Disruption of the micelle formation by the tea catechins prevents the re-absorption of bile acids and thus increasing bile acid excretion, which leads to conversion of cholesterol in the liver to bile acids. Patients treated with tea for 8 weeks were found to have reduced atherogenic index (Yang and Koo, 1997). The resin of *Commiphora mukul* (gugulipid) used in ayurvedic medicine decreased the LDL-C concentration by increasing the uptake and metabolism of LDL-C cholesterol by the liver (Singh et al., 1994). (Hypercholesterolemic patients responded more favorably to gugulipid treatment than hypertriglyceridemic patients (Nityanand et al., 1989).

Grape has been widely used for its cholesterol lowering activity. The grape polyphenols reduce the cholesterol absorption by increasing excretion of fecal bile acids as well as by regulating the expression of LDL-C receptors (Tebib et al., 1994; Pal et al., 2003). Besides, garlic (*Allium sativum*) is a common functional food ingredient that is capable of lowering serum cholesterol levels. Garlic powder (Ali et al., 2000), aged garlic (Yeh and Liu, 2001), raw garlic and boiled garlic (Thomson et al., 2006) have been shown to reduce TC and TG levels along with increase in HDL-C level (Kwon et al., 2003). Allicin, the active ingredient of garlic is able to decrease cholesterol synthesis by inhibiting HMG-CoA reductase and capable of reducing atherosclerosis lesions (Liu and Yeh, 2002; Kwon et al., 2003). The usage of numerous plant-derived hyperlipidemia drugs in the form of compounds or extracts have been increasing worldwide.

2.3 *Garcinia mangostana*

2.3.1 Botanical aspects of *Garcinia mangostana*

Garcinia mangostana is a tropical evergreen tree, which is widespread in Asian countries, especially Malaysia, Thailand, India, and Sri Lanka. It belongs to genus *Garcinia* and family of Clusiaceae. It is a medium tree, 9-18 m tall, with a straight trunk and a rounded, dense crown. All parts of the plant exude yellow latex when wounded. The thick leaves are opposite, elliptical and bright green, ranging from 8-15 cm in length. The flowers are solitary or in pairs at the branch apex and the fruits are round, 5-8 cm in diameter, with a thick purple rind. The pulp is aromatic and has a delightful sweet sour taste. The fruit is known as one of most attractive tropical fruit and named as queen of tropical fruits (Chaivisuthangkura *et al.,* 2009). The plant parts are shown in figure 2.3.

Figure 2.3: *Garcinia mangostana.* (A) Whole plant (B) Stem and bark (C) Flesh, hull, stalk and petals (D) Leaves

2.3.2 Ethnobotanical aspects of *Garcinia mangostana*

G. mangostana has been used as medicine for many years in Southeast Asia. The fruit hull have been used for healing wounds externally, suppurations and chronic ulcers Farnsworth and Bunyapraphatsara, 1992; Saralamp et al., 1996; Nakatani et al.,2002; Moongkarndi et al., 2004; Ji et al., 2007; Yu et al., 2007). In Thailand, the fruit hull has been in applied for the treatment of skin infections, wounds and for the relief of diarrhea (Suksamrarn *et al.*, 2002; Jung *et al.*, 2006).

An ointment derived from the leaves and bark has been used for treating eczema, hyperkeratosis and other skin disorders such as psoriasis (Matsumoto et al., 2003; Sato et al., 2004; Sakagami et al., 2005). In Philippines and Malaysia, decoction of the leaves and bark is used as a febrifuge as well as in the treatment of diarrhea, dysentery and various urinary disorders (Nakatani et al., 2002; Moongkarndi et al., 2004). Besides, the fruit hull decoction has been utilized in relieving diarrhea, cystitis, gonorrhoea, gleet and as astringent lotion (Farnsworth and Bunyapraphatsara, 1992; Moongkarndi et al., 2004; Sato et al., 2004).

In Caribbean and Latin America, tea made from the fruits is used as tonic for fatigue and low energy states whereas similar tea is used to aid in digestion used as digestive relieve in Brazil. Subsequently, the fruit hull has been used to treat parasitic skin infections in Venezuela (Chairungsrilerd et al., 1996; Gopalakrishnan et al., 1997).

2.3.3 Chemical constituents of *Garcinia mangostana*

G. mangostana contains variety of chemicals constituents belonging to the benzophenones, flavonoids, anthocyanins and the major bioactive secondary metabolites are xanthones and its derivatives. The following chemical constituents have been isolated from *G. mangostana*.

2.3.3.a Xanthones

The xanthones isolated from *G. mangostana* are α-mangostin, β-mangostin, γ-mangostin, gartanin, 3-isomagostin,calabaxanthone, 2-(γ,γ-dimethylallyl)-1,7-dihydroxy-3-methoxyxanthone, demethylcalabaxanthone, 1-isomangostin, 3-isomangostin hydrate, 2,8-bis-(γ,γ-Dimethyallyl)-1,3,7-trihydroxyxanthone (Mahabusarakam et al., 1987); 8-deoxygartanin, mangostanol (Chairungsrilerd et al., 1996); 8-hydroxycudraxanthone, garcinone E, mangostingone, tovophyllin, smeathxanthon (Jung et al., 2006); mangostenol, garcinone B, mangostenone, mangostenone A, mangostenone B, mangostenone C, mangostenone D, mangostenone E, (Suksamrarn et al., 2002); 1,2-dihydro-1,8,10-trihydroxy-2-(2-hydroxypropan-2-yl)-9-(3-methylbut-2enyl)furo[3,2-a]xanthen-11-one, 6-Deoxy-7-demethyl mangostanin (Chin et al., 2008); garcimangosxanthone A-C (Zhang et al., 2010); cratoxyxanthone (Wang et al., 2011); E, 11-hydroxy-1-isomangostin (Suksamrarn et al., 2006); mangosharin (Ee et al., 2006); tovophyllin B, garcinone C, garcinone D, 1,7-dihydroxy-2-isopentyl-3-methoxy xanthone, 1,5,8-trihydroxy-3-methoxy-2-(3-methylbut-2-enyl) xanthone, 1,6-dihydroxy-3-methoxy-2-(3-methyl-2-buthenyl)- xanthone,1,3,6,7 tetrahydroxyxanthone, 1,3,8-trihydroxy-4-methyl-2,7-diisoprenylxanthone, 1,5-dihydroxy-2-isopentyl-3-methoxy xanthone (Vieira and Kijjoa, 2005); 1,3-Dihydroxy-2-(2-hydroxy-3-methylbut-3-enyl)-6,7-dimethoxy-8-(3-methylbut-2-enyl)-xanthone, 1,6-dihydroxy-2-(2-hydroxy-3-methylbut-3-enyl)-3,7-dimethoxy-8-(3-methylbut-2-enyl)-xanthone, 1,6-dihydroxy-3,7-dimethoxy-2-(3-methylbut-2-enyl)-8-(2-oxo-3-methylbut-3-enyl)-xanthone, 1,6-dihydroxy-3,7-dimethoxy-2-(3-methylbut-2-enyl)-xanthone, 1,6-dihydroxy-8-(2-hydroxy-3-methylbut-3-enyl)-3,7-dimethoxy-2-(3-methylbut-2-enyl)-xanthone, 1-hydroxy-2-(2-hydroxy-3-methylbut-3-enyl)-3,6,7-trimethoxy-8-(3-methylbut-2-enyl)-xanthone, 1-

hydroxy-8-(2-hydroxy-3-methylbut-3-enyl)-3,6,7-trimethoxy-2-(3-methylbut-2-enyl)-xanthone, mangostanin, garciniafuran, (16E)-1,6-dihydroxy-8-(3-hydroxy-3-methylbut-1-enyl)-3,7-dimethoxy-2-(3-methylbut-2-enyl)-xanthone and (16E)-1-hydroxy-8-(3-hydroxy-3-methylbut-1-enyl)-3,6,7-trimethoxy-2-(3-methylbut-2-enyl)-xanthone (Harrison, 2002).

2.3.3.b Anthocyanins

The anthocyanins isolated from *G.mangostana* are chrysanthemin, cyanidin-3-O-sophoroside and cyanidin-3-O-glucoside (Farnsworth and Bunyapraphatsara ,1992).

2.3.3.c Benzophenones

The benzophenones isolated from *G.mangostana* are garcimangosone D (Huang et al., 2001); kolanone and maclurin (Farnsworth and Bunyapraphatsara, 1992).

2.3.3.d Flavonoids

The flavonoids isolated from *G.mangostana* is epicatehin (Chairungsrilerd et al., 1996; Suksamram et al., 2002; Yu et al., 2007).

2.3.4 Pharmacological activities of *Garcinia mangostana*

Various pharmacological activities have been conducted for *G. mangostana,* where these studies consist of both natural extracts and synthetic derivatives leading to possible therapeutic applications.

2.3.4.a Antioxidant

The antioxidant activity of *G. mangostana* extracts and xanthones were extensively studied using various kinds of assays including ferric thiocyanate, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid (ABTS) free radical scavenging activities (Yoshikawa et al., 1994; Leong and Shui, 2002; Weecharangsan et al., 2006; Chomnawang et al., 2007; Fan and Su, 1997; Haruenkit et al., 2007). Williams et al. (1995) found that α-mangostin protect LDL-C against oxidation induced by metal ion dependent and independent aqueous peroxyl radical. α-mangostin was able to prolonged lag time of conjugated dienes in a dose dependent manner, reduces thiobarbituric reactive substances (TBARS) production, and decreases utilization of α-tocopherol induced by LDL-C oxidation. Moreover, α-mangostin exhibited protective effect against antioxidant defense system and lipid peroxidation in injury-induced myocardial infarction rats (Devi Sampath and Vijayaraghavan, 2007). The antioxidant properties of *G. mangostana* extracts and xanthones have been summarized in table 2.2.

Table 2.2: Antioxidant properties of *Garcinia mangostana*

Extracts of *G. mangostana* and xanthones	**References**
The methanol extract of the fruit hulls of *G. mangostana* showed DPPH scavenging activity	Yoshikawa et al., 1994
α-mangostin inhibited copper-induced LDL-C oxidation *in vitro*	Williams et al., 1995
α and γ -mangostin showed antioxidant activity using the ferric thiocyanate method	Fan and Su,1997
Methanolic extract of the edible portion of *G. mangostana* exhibited antioxidant activity in DPPH and ABTS assays	Leong and Shui, 2002
The aqueous and ethanolic extracts of the hul of *G. mangostana* exhibited DPPH scavenging activity and protects neuroblastoma cell line NG108-15 from H_2O_2 cytotoxicity	Weecharangsan et al., 2006
The ethanolic extract of *G. mangostana* showed antioxidant activity against DPPH radicals and reduced the ROS production of polymorphonuclear leukocytes (PML)	Chomnawang et al., 2007
Mangosteen fruit showed antioxidant activity against DPPH and ABTS radicals and prevents the decrease in antioxidant activity induced by a cholesterol supplemented diet in rats	Haruenkit et al., 2007
α-mangostin showed protective effect against isoproterenol-induced oxidative damage and myocardial injury in rats	Devi Sampath and Vijayaraghavan, 2007

2.3.4.b Antitumor

Six xanthones were isolated from fruit hull of *G. mangostana* and Garcinone E was found to be the most toxic xanthone among them. Garcinone E exhibited potent cytotoxic effect on HCC36, TONG, HA22T, Hep3B, HEpG2 and SK-Hep-1 hepatocellular carcinoma cell lines, NCIHut 125, CH27 LC-1, H289, Calu-1 lung carcinoma cell lines and AZ521, NUGC-3, KATO-III and AGS gastric carcinoma cell lines (Ho et al., 2002). Garcinone E lethal dose 50% (LD50) was between 0.1 and 5.4 μM and showed a wide range of dose as well as time dependent effects against the cell lines.

On the other hand, α, β, γ-mangostins, mangostinone, garcinone E and 2-isoprenyl- 1,7-dihydroxy-3-methoxy xanthone from the fruit hull were tested on the cell growth inhibition of human leukemia cell line HL60. The cytotoxic effect tested for 72 hrs after cell incubation with xanthone at 5 or 40 μM whereby xanthones showed significant inhibition. α, β, and γ-mangostin exhibited cytotoxicity from 10 μM and α-mangostin showed the highest inhibitory activity with IC_{50} of 10 μM (Matsumoto et al., 2003). In another study, the aqueous extract of *G. mangostana* fruit hull showed effective anti leukemia activity with IC_{50} of 61 ± 9.9 and 159 ± 12 μg/mL against K562 and Raji leukemia cells, respectively compared to seventeen most used fruits in Taiwan. The aqueous extract also had a moderate activity against U937 leukemia cells, and less effective against P3HR1 leukemia cells (Chiang et al., 2004).

Nabandith et al. (2004) reported that dietary administration of α-mangostin showed significant short-term chemo preventive inhibition on putative preneoplastic lesions in rat colon carcinogenesis, induced by subcutaneous injection of 1,2-dimethylhydrazine, DMH (40 mg/kg). Meanwhile, Matsumoto et al. (2005)