PHENOLIC ACID RICH FRACTION OF *GYNURA PROCUMBENS* AS POTENTIAL ANTIHYPERLIPIDEMIC AND ANTIOXIDANT AGENTS

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by

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LIST OF ABBREVIATIONS

1,3DC	1,3-dicaffeoylquinic acid
1,5DC	1,5-dicaffeoylquinic acid
3,4DC	3,4-dicaffeoylquinic acid
3,5DC	3,5-dicaffeoylquinic acid
4,5DC	4,5-dicaffeoylquinic acid
^{13}C	Carbon-13
^{1}H	Proton
AAW	Acetic acid in water
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
ACN	Acetonitrile
AI	Atherogenic index
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
Apo	Apolipoprotein
ARASC	Animal Research and Service Centre
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
CA	Chlorogenic acid
CE	Catechin equivalent
CETP	Cholesterol ester transfer protein
cm	Centimeter
cm^{-1}	Unit for wavenumber
CMC	Carboxymethylcellulose
CO_2	Carbon dioxide
CoA	Coenzyme A
CRI	Coronary risk index
CYP51	Lanosterol 14α-demethylase
CYP7A1	Cholesterol 7α-hydroxylase
d	Doublet
DAD	Diode array detector
dd	Doublet of doublets
DHCR	Dehydrocholesterol reductase
dL	Deciliter
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
Eahy	Human endothelial cells
ELISA	Enzyme-linked immunosorbent assay
Ethanolic extract	95 % ethanolic extract
F1	Water fraction
F2	50 % methanolic fraction
F3	Acetone fraction
FAS	Fatty acid synthase

FAW	Formic acid in water
$FeSO_4$	Ferrous sulphate
FPP	Farnesyl pyrophosphate
FRAP	Ferric reducing antioxidant power
g	Gram
GAE	Gallic acid equivalent
GC	Gas chromatography
GPP	Geranyl pyrophosphate
h	Hour
H&E	Hematoxylin and Eosin
HaCat	Human keratinocyte
HCl	Hydrochloric acid
HCT-116	Human colon cancer cells
HDL-C	High-density lipoprotein-cholesterol
HeLa	Human cervical cancer cells
HepG2 cells	Human liver carcinoma cells
HFD	High fat diet
HILIC	Hydrophilic Interaction Liquid Chromatography
HMG-CoA	3-hydroxy-3-methylglutaryl-coenzyme A
HPLC	High-performance liquid chromatography
HSV	Herpes simplex virus
Hz	Hertz
i.p.	Intraperitoneal
i.v	Intravenous
IC_{50}	Half maximal inhibitory concentration
ICH	International Council for Harmonization
IDL-C	Intermediate-density lipoprotein-cholesterol
IPP	Isopentenyl-diphosphate delta
IR	Infrared spectroscopy
IU	International unit
J	Coupling constant in Hertz
kg	Kilogram
L	Liter
LCAT	Lecithin–cholesterol acyltransferase
LD_{50}	Lethal dose
LDL-C	Low-density lipoprotein-cholesterol
LOD	Limit of detection
LOQ	Limit of quantification
LPL	Lipoprotein lipase
M	Molar
m	Multiplet
m/z	Mass-to-charge ratio
MCF-7	Human breast cancer cells
MeOH	Methanol
mg	Milligram
min	Minutes
mL	Milliliter

mm	Millimeter
mM	Millimolar
mmol	Millimoles
mol	Moles
MS	Mass spectrometry
MTT	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H tetrazolium salt
MW	Molecular weight
NADH	Nicotinamide adenine dinucleotide
$NADP^+$	Oxidized nicotinamide adenine dinucleotide phosphate
NADPH	Nicotinamide adenine dinucleotide phosphate
NF-κb	Nuclear Factor Kappa B
nm	Nanometer
NMR	Nuclear magnetic resonance
NP-PEG	Natural product-polyethylene glycol
O.D.	Optical density
OECD	Organization for Economic Cooperation and Development
p	Probability value
p-407	Poloxamer-407
pH	Potential of hydrogen
PPARα	Peroxisome proliferator-activated receptor alpha
ppm	Parts per million
PTFE	Polytetrafluoroethylene
QE	Quercetin equivalent
ROS	Reactive oxygen species
RP	Reverse phase
rpm	Revolutions per minute
RPMI	Roswell Park Memorial Institute
RSD	Relative standard deviation
S(ethanolic extract)	Standardized ethanolic extract
S(F1)	Standardized F1
S(F2)	Standardized F2
S(F3)	Standardized F3
SD	Sprague Dawley
sec	Seconds
SEM	Standard error of mean
s-ICAM-1	Soluble-intercellular adhesion molecule 1
SPE	Solid phase extraction
SPSS	Statistical Package for the Social Sciences
s-VCAM-1	Soluble-vascular cell adhesion molecule 1
T cells	T helper cell
TC	Total cholesterol
td	Triplet of doublets
TEAC	Trolox equivalent antioxidant capacity
TFC	Total flavonoid content
TG	Triglycerides
TLC	Thin layer chromatography

TPC	Total phenolic content
TPTZ	2,4,6-Tris(2-pyridyl)-s-triazine
U	Unit
UV-vis	Ultraviolet–visible
v/v	Volume over volume
VLDL-C	Very low-density lipoprotein-cholesterol
w/v	Weight over volume
w/w	Weight over weight
WHO	World Health Organization

LIST OF SYMBOLS

α	Alpha
Å	Angström
β	Beta
^{0}C	Degree Celsius
°E	East
γ	Gamma
λ	Lambda
°N	North
π	Pi
μ	Micro
<	Less than
>	More than

FRAKSI *GYNURA PROCUMBENS* YANG DIPERKAYA DENGAN ASID FENOLIK SEBAGAI AGEN ANTIHIPERLIPIDEMIK DAN ANTIOKSIDA BERPOTENSI

ABSTRAK

G. procumbens boleh didapati di kebanyakan negara Asia Tenggara dan daunnya digunakan sebagai ubat untuk merawat pelbagai penyakit termasuk hiperlipidemia. Objektif kajian ini termasuk pemencilan sebatian penanda, pemiawaian dan analisis fitokimia sampel tumbuhan *G. procumbens* dan penyelidikan kesan antioksida dan antihiperlipidemik mereka dalam model tikus akut dan kronik, penentuan tindakan mekanisma dan profil toksikologi mereka. Sampel daun *G. procumbens* telah diekstrak dengan 95 % etanol dan difraksinasi kepada tiga fraksi: F1 (air), F2 (50 % metanol) dan F3 (aseton). Pemurnian selanjutnya terhadap F2 telah menghasilkan asid fenolik, asid klorogenik (CA). Analisis antioksida mendedahkan bahawa F2 mempunyai kandungan fenolik tertinggi dan aktiviti antioksida yang kuat. CA juga menunjukkan aktiviti antioksida yang kuat setanding dengan piawaian rujukan. Sampel-sampel *G. procumbens*, ekstrak dan fraksi-fraksi dipiawaikan menggunakan asid fenolik (asid kafioyilkuinik: CA, 3,4DC, 3,5DC dan 4,5DC) sebagai sebatian penanda dengan kaedah RP-HPLC yang disahkan. Analisis fitokimia mendedahkan bahawa, F2 diperkaya dengan asid kafioyilkuinik berbanding dengan fraksi-fraksi lain. Kajian antihiperlipidemik akut ekstrak, fraksi-fraksi dan CA *G. procumbens* yang telah dipiawaikan menyatakan bahawa S(F2) dan CA mempunyai aktiviti antihiperlipidemik yang paling kuat untuk merendahkan tahap TC dan TG dalam tikus hiperlipidemik akut diinduksi dengan p-407 (500 mg/kg; ip). Di samping itu, S(F2) juga mengurangkan

tahap LDL-C, VLDL-C, AI dan CRI secara ketara selepas rawatan selama 58 jam diikuti oleh CA. Oleh itu, S(F2) telah dipilih untuk penyiasatan lanjut menggunakan model hiperlipidemik kronik. Rawatan selama lima minggu dengan pelbagai dos S(F2) menghasilkan kesan antihiperlipidemik bersandarkan dos, di mana kesan yang paling kuat diberikan oleh 500 mg/kg S(F2). Fraksi tersebut didapati merendahkan tahap TC, TG, LDL-C, VLDL-C, CRI dan AI pada masa yang sama meningkatkan paras HDL-C tikus hiperlipidemik secara ketara. Kedua-dua S(F2) dan CA telah menunjukkan aktiviti perencatan pada enzim HMG-CoA reduktase sementara, hanya CA mempamerkan aktiviti perencatan sederhana terhadap enzim lipase pankreas. Di samping itu, S(F2) menunjukkan keberkesanan bersandarkan dos dimana dos 500 mg/kg menurunkan tahap sintesis lipid hati (TC dan TG) dan meningkatkan perkumuhan lipid (TC) dan asid hempedu melalui tinja. CA menunjukkan kesan sitotoksik sederhana terhadap MCF-7 dan Hela, manakala ia adalah selamat pada sel-sel HCT-116 dan Eahy. Sebaliknya, S(F2) tidak menunjukkan kesan sitotoksik pada semua sel kanser dan normal. Kajian toksisiti akut pada S(F2) menunjukkan bahawa fraksi tersebut adalah selamat dan LD_{50} dianggarkan lebih daripada 5000 mg/kg. Kesimpulannya, fraksi bioaktif S(F2) berpotensi sebagai agen merendahkan lipid yang boleh menghalang hiperlipidemia dan penyakit kardiovaskular dengan mengurangkan tahap lipid serum.

PHENOLIC ACID RICH FRACTION OF *GYNURA PROCUMBENS* AS POTENTIAL ANTIHYPERLIPIDEMIC AND ANTIOXIDANT AGENTS

ABSTRACT

G. procumbens is found in most of the Southeast Asian countries and the leaves are used as a folk medicine to treat various illnesses including hyperlipidemia. The objectives of the present study include isolation of marker compound(s), standardization and phytochemical analysis of *G. procumbens* plant samples and investigation of their antioxidant and antihyperlipidemic effects in acute and chronic rat models, determination of their mechanisms of action and toxicological profiles. The leaves of *G. procumbens* were macerated with 95 % ethanol and fractionated into three fractions: F1 (water), F2 (50 % MeOH) and F3 (acetone). Further purification of F2 yielded a phenolic acid, chlorogenic acid (CA). Antioxidant analyses revealed that, F2 possessed highest phenolics content and strong antioxidant activities. Likewise, CA exhibited potent antioxidant activities that were comparable to reference standards. The *G. procumbens* plant samples, extract and fractions were standardized using phenolic acids (caffeoylquinic acids: CA, 3,4DC, 3,5DC and 4,5DC) as marker compounds by a validated RP-HPLC method. Phytochemical analysis revealed that, F2 was enriched with caffeoylquinic acids compared to other fractions. Acute antihyperlipidemic study of standardized extract, fractions and CA of *G. procumbens* indicated that S(F2) and CA had most potent antihyperlipidemic activity on lowering TC and TG levels in p-407 (500 mg/kg; i.p.) induced acute hyperlipidemic rats. In addition, S(F2) also significantly reduced levels of LDL-C, VLDL-C, AI and CRI after 58 h treatment followed by CA.

Hence S(F2) was chosen for further investigations in chronic hyperlipidemic model. Five weeks of treatment with various doses of S(F2) resulted in dose dependent antihyperlipidemic effects, whereby the most potent effect was exerted by 500 mg/kg of S(F2). The fraction significantly decreased TC, TG, LDL-C, VLDL-C, CRI and AI levels while increased HDL-C level of hyperlipidemic rats. S(F2) and CA showed inhibitory effect on HMG-CoA reductase enzyme while, only CA had moderate inhibitory activity on pancreatic lipase enzyme. In addition, S(F2) (500 mg/kg) dose dependently reduced liver lipids synthesis (TC and TG) and increased the excretion of lipids (TC) and bile acids via feces. CA showed mild cytotoxic effects against MCF-7 and Hela, while it was safe on HCT-116 and Eahy cells. In contrast, S(F2) exhibited no cytotoxic effects on all cancer and normal cell lines. Acute toxicity on S(F2) indicated that the fraction was safe and LD_{50} was greater than 5000 mg/kg. In conclusion, S(F2) bioactive fraction is a potential lipid-lowering agent which may prevent hyperlipidemia and cardiovascular diseases by lowering serum lipid levels.

CHAPTER 1

INTRODUCTION

1.1 Background

Around the world, non-communicable diseases such as cardiovascular diseases, cancer, diabetes and chronic respiratory diseases kill 40 million people each year (WHO, 2017). Health data gathered from countries around the world revealed that cardiovascular diseases remain the leading cause of death with ≈17.7 million deaths annually (WHO, 2017). In Malaysia, 73 % of total deaths are contributed by non-communicable diseases with cardiovascular diseases being the main contributor (NHMS, 2015). There are a number of risk factors associated with the development of cardiovascular diseases such as hyperlipidemia, obesity, hypertension and diabetes which are caused by unhealthy lifestyle factors which include smoking, unhealthy diet and lack of physical exercises (Buttar et al., 2005). Among the risk factors, hyperlipidemia plays a significant role in inducing atherosclerosis which leads to heart attack, stroke, angina and various types of heart and blood vessel disorders, collectively belong to cardiovascular diseases (WHO, 2017; Kaur et al., 2013).

Hyperlipidemia is defined as elevated levels of blood total cholesterol (TC), triglycerides (TG), low-density lipoprotein-cholesterol (LDL-C) and declining of high-density lipoprotein-cholesterol (HDL-C). WHO (2017) revealed that hyperlipidemia associated diseases cause approximately 2.6 million deaths annually worldwide and the prevalence is the highest in Europe with 54 % for both sexes. While in Malaysia, NHMS

(2015) revealed that 47.7 % of total population aged above 18 is suffering from this condition, which has doubled since 2006 (20.7 %). The incidence of hyperlipidemia is known to be one of the main contributors for cardiovascular diseases in Malaysia (NHMS, 2015). Despite the availability of various antihyperlipidemic drugs (statins, niacin, fibrates, bile acid sequestrants) in the market, many still opt for herbal medicines as an alternative. The demand and popularity for herbal medicines in both developed and developing countries are due to the presumed minor side effects, good efficacy in management of human diseases and their availability at affordable prices (Gunjan et al., 2015).

Herbs or medicinal plants usually have broad pharmacological activities mainly due to their bioactive phytochemicals such as the flavonoids, alkaloids, terpenoids, glycosides and lignans. In addition to their medicinal properties, medicinal plants are also well known for their antioxidant potential to fight against reactive oxygen species (ROS) which is the main contributor to the development of many diseases (Chodak et al., 2011). Of interest, a particular attention has been focused on phenolic acids especially mono- and di-caffeoylquinic acids due to their extended range of pharmacological activities besides their known antioxidant and radical scavenging activities (Križman et al., 2007).

Numerous studies on various plants have revealed that consumption of polyphenols/phenolic acids rich plants may reduce the risk of cardiovascular diseases caused by hyperlipidemia via lipid lowering mechanism (Križman et al., 2007; Inbaraj et al., 2010). Moreover, literature have also confirmed that medicinal plants having both

antihyperlipidemic and antioxidant properties are better in managing disorders associated with hyperlipidemia which are usually accompanied by increased oxidative stress (Patil et al., 2014). Studies also have revealed that caffeic acid derivatives such as 1,3-dicaffeoylquinic acid (1,3DC), 1,5-dicaffeoylquinic acid (1,5DC), 3,4-dicaffeoylquinic acid (3,4DC), 3,5-dicaffeoylquinic acid (3,5DC) and 4,5-dicaffeoylquinic acid (4,5DC) possessed better antioxidant potential and displayed protective role against cardiovascular diseases nearly tenfold higher than normal mono-caffeoylquinic acids (Chen et al., 2013; Wang et al., 2009; Inbaraj et al., 2010). Considering all the factors mentioned above, it is rationale to investigate a plant that has been in existence in the traditional medicinal system for a long time and is used for management of various human diseases.

Gynura procumbens (Lour.) Merr from Asteraceae family is locally known as 'Sambung nyawa' or 'Bai bing cha' by the Malay and Chinese communities respectively (Shwter et al., 2014). This decumbent perennial herbaceous shrub is abundantly distributed in South East Asian countries such as Malaysia, Thailand, Indonesia, Philippines, China and Borneo (Kaewseejan & Siriamornpun, 2015). Traditionally, the leaves of this plant are used to treat ailments such as fever, migraine, inflammation, kidney problems, rheumatism, diabetes, high blood pressure, rashes and constipation (Kaewseejan & Siriamornpun, 2015; Kaewseejan et al., 2015; Wu et al., 2011). The plant is non-toxic and the leaves are still being used as food source by the natives where it is generally consumed raw as salad. Several studies have reported that the leaves of *G. procumbens* possessed a vast range of pharmacological activities such as antioxidant, antifungal, antiherpes simplex virus, antihyperglycemic, antibacterial, anti-

inflammatory, antiulcer, anticancer and antihypertensive (Wu et al., 2011; Abrika et al., 2013; Iskander et al., 2002; Kaewseejan et al., 2012; Mahmood et al., 2010; Mustafa et al., 2010; Rahman & Asad, 2013). Bioactive chemical constituents isolated from *G. procumbens* leaves include flavonoids, phenolic acids (gallic acid, p-coumaric and ferulic acids), tannins, terpenoids, saponins, alkaloids, coumarins, anthocyanins and stigmasterol (Kaewseejan & Siriamornpun, 2015; Rahman & Asad, 2013).

1.2 Problem statement

Previous studies reported that 95 % ethanolic extract (ethanolic extract) of *G. procumbens* leaves contained high contents of phenolics and flavonoids (Kaewseejan & Siriamornpun, 2015). However, not many studies were carried out to determine the classes/individual compounds or fractions of *G. procumbens* extracts that are responsible for its pharmacological activities. Zhang and Tan (2000), first reported on the lipid lowering effect of ethanolic extract of *G. procumbens* in streptozotocin-induced diabetic rats. Preliminary findings by Meng (2011) and Saeed (2013) revealed that the ethanolic extract of *G. procumbens* had promising antihyperlipidemic effect by reducing blood lipid levels of the acute and chronic hyperlipidemic rat models. However, to date, a question still remains on which class(es) of chemical constituent(s) or compound(s) is/are responsible for the antihyperlipidemic activity observed by the earlier researchers. Meng (2011) has also reported on the isolation of two major phenolic acids from the ethanolic extract of *G. procumbens*, namely chlorogenic acid (CA) and 3,5DC. This has created an interest to work on the bioactivity of the fractions of ethanolic extract of *G. procumbens* to evaluate their antihyperlipidemic effects and further investigate the possible mechanisms of action and toxicity.

1.3 Hypothesis

Phenolic acid rich fraction is expected to be fractionated from the ethanolic extract of *G. procumbens* using resin column technology. Phenolic acid(s) will then be isolated from the bioactive fraction and the isolated compound(s) will be used as marker compounds to standardize *G. procumbens* plant samples. The phenolic acid rich bioactive fraction is expected to possess potent antioxidant and antihyperlipidemic activities. Hence, the fraction and the isolated compound(s) should be able to cure hyperlipidemia via multiple mechanisms such as inhibition of lipid related enzymes, increased secretion of fecal bile acids and excretion of lipids. The bioactive fraction and isolated compound(s) are also expected to be safe for ingestion.

1.4 Research objectives

The main objective of the present study is to evaluate antihyperlipidemic activity of standardized phenolic acid rich fraction of *G. procumbens* using animal models and to investigate the mechanisms of its antihyperlipidemic action.

The sub-objectives of present study include:

1. To fractionate the ethanolic extract of *G. procumbens* using resin column technology.
2. To isolate and characterize phenolic acid(s) from ethanolic extract of *G. procumbens*.
3. To evaluate antioxidant activities of ethanolic extract of *G. procumbens* and its fractions and phenolic acid(s).

4. To standardize ethanolic extract of *G. procumbens* and its fractions using phenolic acid(s) isolated as marker compounds.
5. To evaluate antihyperlipidemic activities of standardized ethanolic extract of *G. procumbens* and its fractions and phenolic acid(s) in chemically-induced hyperlipidemic rats.
6. To evaluate antihyperlipidemic activity of standardized bioactive fraction of *G. procumbens* in high fat diet (HFD)-induced hyperlipidemic rats.
7. To investigate the mechanisms of antihyperlipidemic activity of standardized bioactive fraction of *G. procumbens* and its phenolic acid(s) on:
    a) inhibition of selected enzymes involved in lipids biosynthesis and metabolism
    b) removal of excess lipids through bile acids and feces
8. To investigate the potential cytotoxicity and acute toxicity of the standardized bioactive fraction of *G. procumbens* and its phenolic acid(s).

The research flow is summarized in Figure 1.1.

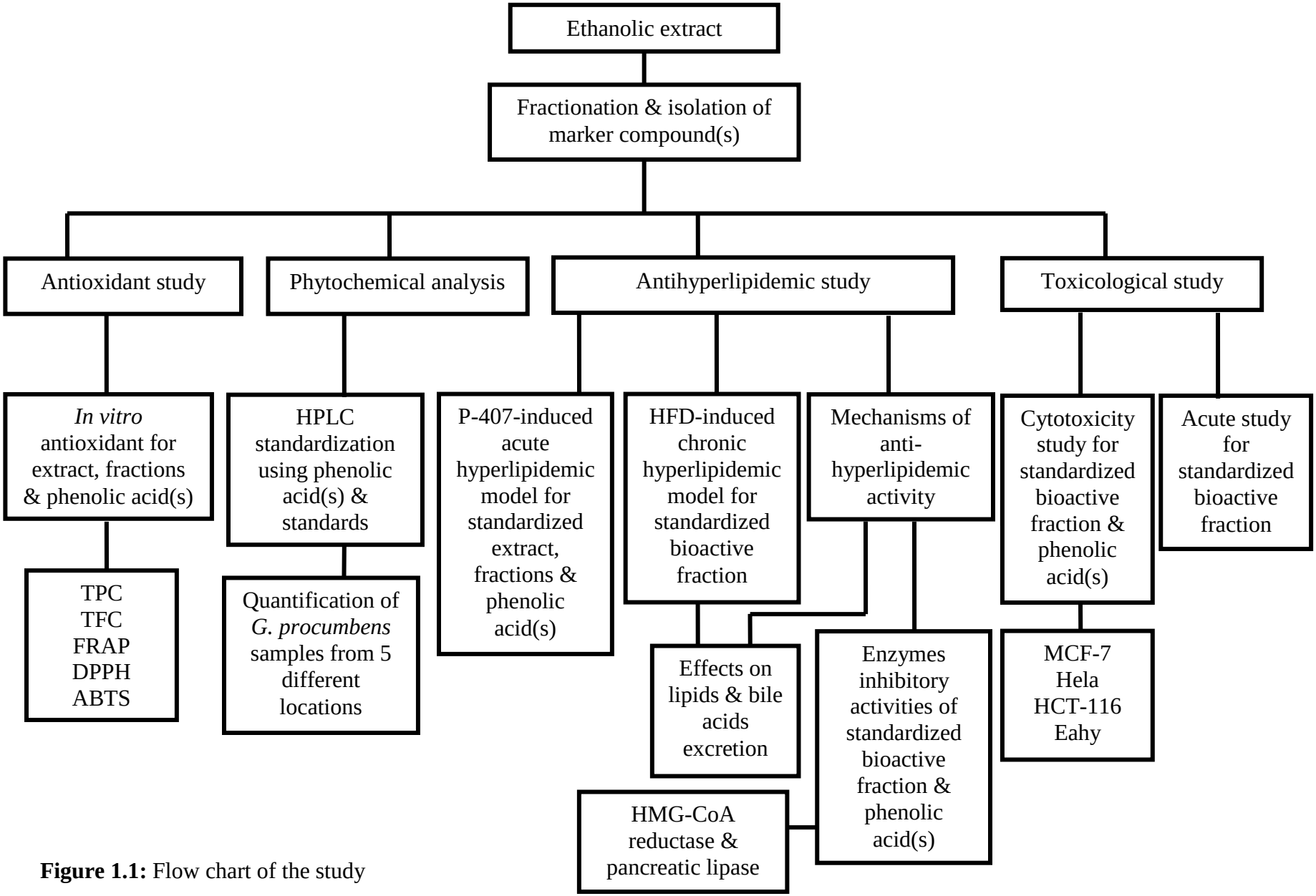


Figure 1.1: Flow chart of the study

CHAPTER 2

LITERATURE REVIEW

2.1 The genus *Gynura*

The genus *Gynura* was first described by Cassini in 1825 and so far forty four species have been identified globally (Vanijajiva & Kadereit, 2011). *Gynura* is a perennial herbaceous shrub that belongs to Asteraceae-Senecioneae family which is widely distributed in tropical countries such as Africa, Southeast Asia, Southern Japan, Southern China, Northern Australia and New Guinea (Kaewseejan & Siriamornpun, 2015). About hundreds of research papers have been published on the phytochemical and pharmacological studies of the genus, *Gynura*. Among the species that have been studied are *G. angulosa*, *G. aurantiaca*, *G. bicolor*, *G. calciphila*, G. *crepidioides, G. cusimbua*, *G. divaricata*, *G. elliptica*, *G. formosana*, *G. japonica*, *G. medica*, *G. nepalensis*, *G. pseudochina*, *G. scandens* and *G. segetum* (Vanijajiva & Kadereit, 2011).

2.2 *Gynura procumbens* (Lour.) Merr.

2.2.1 Description of *Gynura procumbens* (Lour.) Merr.

G. procumbens (Figure 2.1) is an evergreen plant belonging to the Astereceae (Compositae) family indigenous to Southeast Asia especially Malaysia, Indonesia and Thailand (Bhore et al., 2010). In Malaysia, this plant species can be found growing wild or cultivated and has limited distribution at the western part of peninsular Malaysia (Keng et al., 2009). The plant has several scientific synonyms such as *Cacalia*

procumbens Lour., *Calacia procumbens* Lour., *G. sarmentosa* DC. and *Cacalia sarmentosa Blume* (Mustaffa et al., 2011). Taxonomic classification of *G. procumbens* is shown in Table 2.1.

Figure 2.1: *Gynura procumbens* plant

Table 2.1: Taxonomic classification of *Gynura procumbens* (Lour.) Merr.

Domain	Eukaryota
Kingdom	Plantae
Subkingdom	Viridiplantae
Class	Spermatophyta
Order	Asterales
Family	Asteraceae
Subfamily	Asteroideae
Genus	*Gynura*
Species	*procumbens*
Scientific name	*Gynura procumbens*

G. procumbens is commonly known as sambung nyawa (prolongation of life), daun dewa, akar sebiak or kecam akar by the Malays while ‘bai bing cha’ (100 ailments) by the Chinese in Malaysia (Tan et al., 2016). The reason why the plant is called sambung nyawa and ‘bai bing cha’ is due to the vast range of uses in traditional medicine. Preparations of *G. procumbens* have been utilized traditionally for the treatment of skin conditions, kidney problems, rheumatism, hypertension, diabetes, viral infection, ringworm infection, constipation, inflammation, eruptive fever, rashes, migraine and cancer (Bhore et al., 2010; Mustaffa et al., 2011; Tan et al., 2016).

Even the leaves of this plant have been used extensively in different ways as a part of food in different cultures. In Malaysia, the fresh leaves are edible as salad known as ‘ulam’, eaten with rice and used as flavoring for food. Meanwhile in Thailand, the leaves are either eaten raw or boiled to be used as a garnish in food preparations such as curries, salads, soups and entrees. Scientific investigations on the leaves of this plant have shown that it’s safe for consumption (Rosidah et al., 2009).

G. procumbens is a fast growing decumbent shrub that can grow up to 10-25 cm tall (Keng et al., 2009). The plant is highly branched with hairy leaves arranged alternately on hairy stem (Rahman & Asad, 2013; Saiman et al., 2012; Keng et al., 2009; Mustaffa et al., 2011). The leaves of this species normally are green in color, succulent, hairy on both surfaces, differ in shape either ovate, elliptic or lanceolate, 3.5-8.0 cm long, 0.8–3.5 cm wide, with cuneate or rounded base and an acute or obtuse attenuated apex (Rahman & Asad, 2013; Li et al., 2015; Keng et al., 2009; Mustaffa et al., 2011). The stem of the plant is fleshy and has purple tint. The flowering heads are 1–1.5 cm

long, yellow in color, narrow and panicled (Rahman & Asad, 2013). The plant produces purple flowers that are tubular and bisexual (Sunarwidhi et al., 2014; Keng et al., 2009).

2.2.2 Chemical constituents of *Gynura procumbens* (Lour.) Merr.

In 1996, Sadikun and the co-workers isolated a mixture of sterol and sterol glycosides from the leaves of *G. procumbens.* Flash column chromatography of petroleum ether extract, followed by further purification using preparative thin layer chromatography (TLC) yielded mixture of *β*-sitosterol [1] and stigmasterol [2]. In addition, the chloroform extract subjected to silica gel column chromatography afforded two more compounds 3-*O*-*β*-D-glucopyranosyl *β*-sitosterol [3] and 3-*O*-*β*-D-glucopyranosyl stigmasterol [4]. The group also isolated a nucleic acid, adenosine [5] and two flavanol glycosides, kaempferol-3-*O*-*α*-L-rhamnosyl-(1→2)-O-*β*-D-glucopyranoside [6] and kaempferol-3-*O*-*β*-D-glucopyranoside [7] (Sadikun et al., 1996).

Two types of di-caffeoylquinic acids, 3,5DC [8] and 4,5DC [9] have been identified by Jiratchariyakul et al. (2000) from *G. procumbens* which was found to inhibit the replication of herpes causing viruses. Later, Akowuah et al. (2002) isolated four compounds from the *n*-butanol fraction of petroleum ether extract of *G. procumbens* leaves, namely quercetin-3-*O*-rhamnosyl (1→6) glucoside [10], kaempferol-3-*O*-rhamnosyl (1→6) glucoside [11], kaempferol-3-*O*-glucoside [7] and quercetin-3-*O*-rhamnosyl (1→2) galactoside [12].

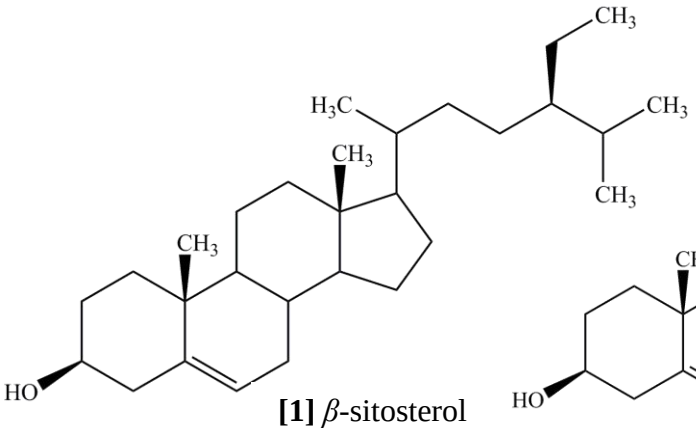
High-performance TLC (HPTLC) analysis of *G. procumbens* methanolic extract and its fractions resulted in identification of two phenolics namely, kaempferol-3-*O*-

rutinoside [11] and astragalin [7] (Rosidah et al., 2008). Nine types of phenolic acids were identified using reverse phase-high-performance liquid chromatography (RP-HPLC) analysis in the ethanolic extract, ethyl acetate fraction and its sub-fractions fractionated using Sephadex LH-20 column chromatography. The phenolic acids identified were, hydroxybenzoic acids: *p*-hydroxybenzoic acid [13], gallic acid [14], protocatechuic acid [15], vanillic acid [16], syringic acid [17] and hydroxycinnamic acids: caffeic acid [18], *p*-coumaric acid [19], ferulic acid [20] and sinapic acid [21] (Kaewseejan et al., 2015).

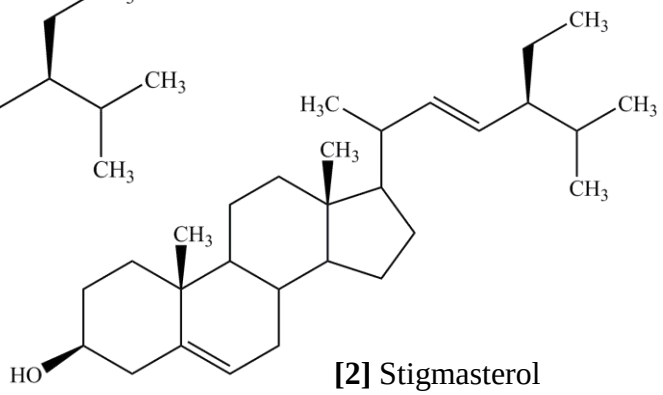
The aerial parts of *G. procumbens* were extracted according to polarity using petroleum ether, dichloromethane and ethanol. The ethanolic extract which showed virucidal and antireplicative actions against herpes simplex virus-1 (HSV-1) and HSV-2 upon further purification afforded kaempferyl-3-*O*-α-L-rhamnosyl(1→6)-β-D-glucopyranoside [11], 3,5DC [8], kaempferyl glucopyranoside [7], 4,5DC [9], kaempferyl-3-*O*-α-L-rhamnosyl(1→6)-β-D-galactopyranoside [22], CA [23], quercetin-3-*O*-β-D-glucopyranoside [24], kaempferol [25], β-sitosterol [1], stigmasterol [2], β-sitosteryl glucoside [3], stigmasteryl glucoside [4] and 1,2-bis-dodecanoyl-3-α-D-glucopyranosyl-sn-glycerol [26] (Jarikasem et al., 2013).

Phytochemical analysis of the antihyperglycemic active fraction using HPLC revealed the presence of kaempferol-3,7-di-*O*-β-D-glucoside [27], which was shown to be responsible for antihyperglycemic activity of ethanolic extract of *G. procumbens* (June et al., 2012). Zhang and co-workers investigated methanol (MeOH) extract of the

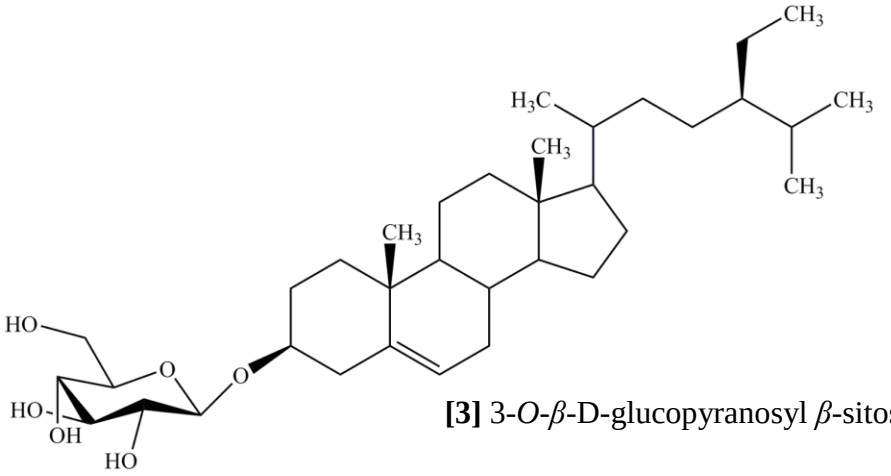
leaves of *G. procumbens* which afforded a new sesquiterpenoid, muurol-4-ene-1*β*, 3*β*, 10*β*-triol [28], two sesquiterpene glycosides, muurol-4-ene-1*β*,3*β*,10*β*-triol 3-*O*-*β*-D-glucopyranoside [29] and muurol-4-ene-1*β*,3*β*,15-triol 3-*O*-*β*-D-glucopyranoside [30] and three known sesquiterpenoids, schensianol A [31], negunfurol [32], and 4 *β*,10*α*-aromadendranediol [33] (Zhang et al., 2014b).



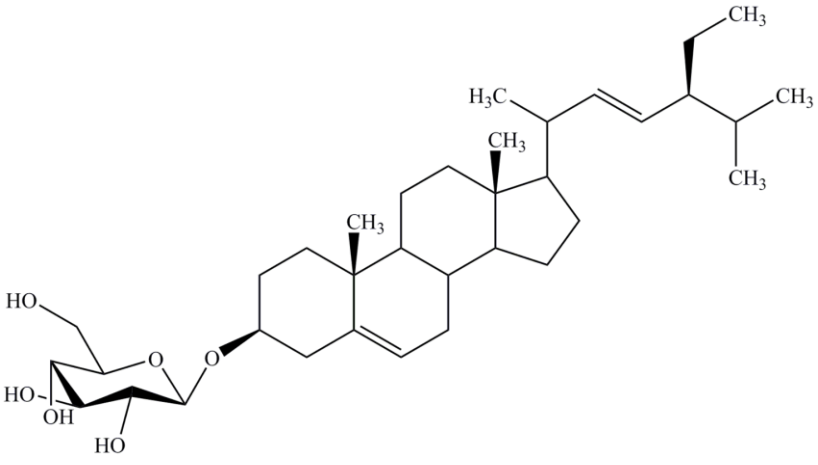
[1] *β*-sitosterol



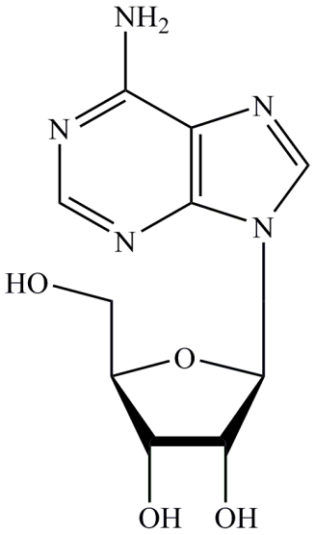
[2] Stigmasterol



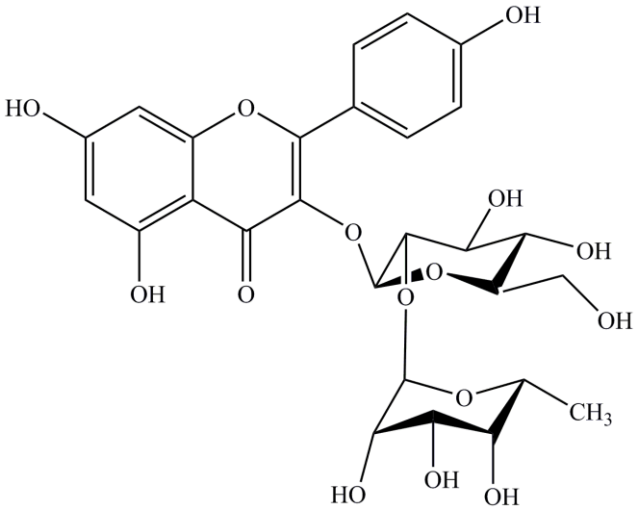
[3] 3-*O*-*β*-D-glucopyranosyl *β*-sitosterol



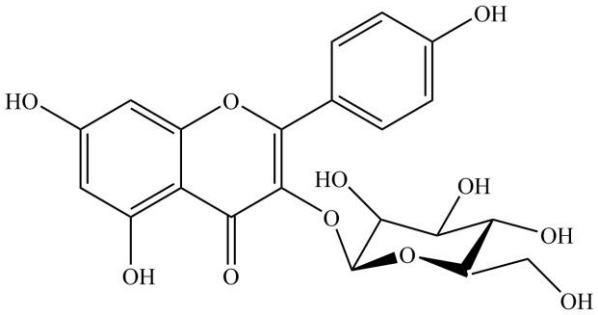
[4] 3-*O*-*β*-D-glucopyranosyl stigmasterol



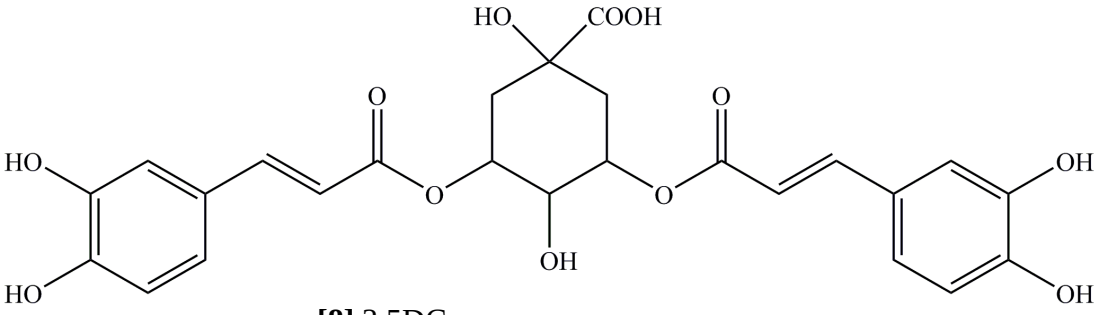
[5] Adenosine



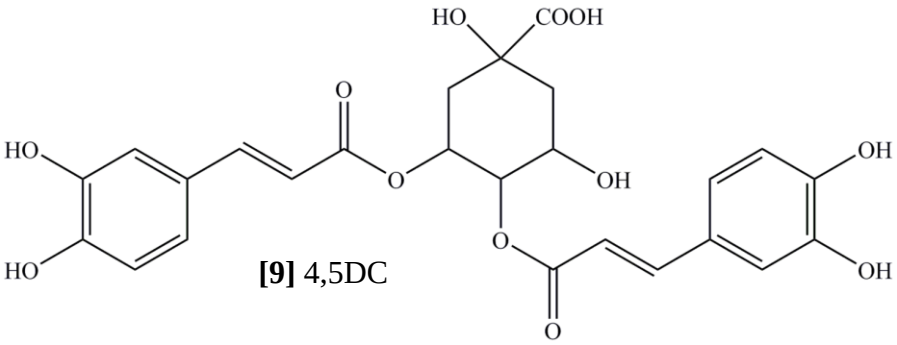
[6] Kaempferol-3-*O*-*α*-L-rhamnosyl-(1→2)-O-*β*-D-glucopyranoside



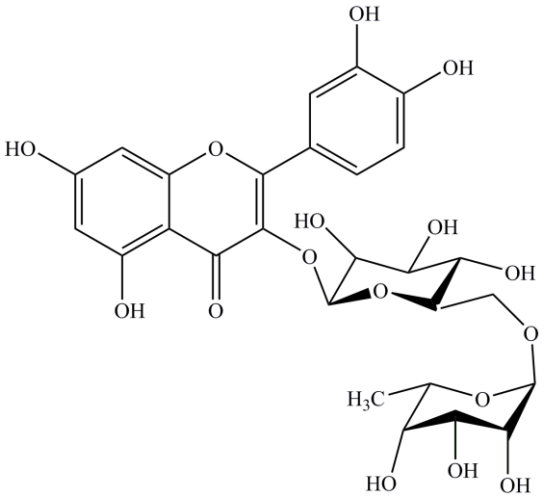
[7] Kaempferol-3-*O*-*β*-D-glucopyranoside



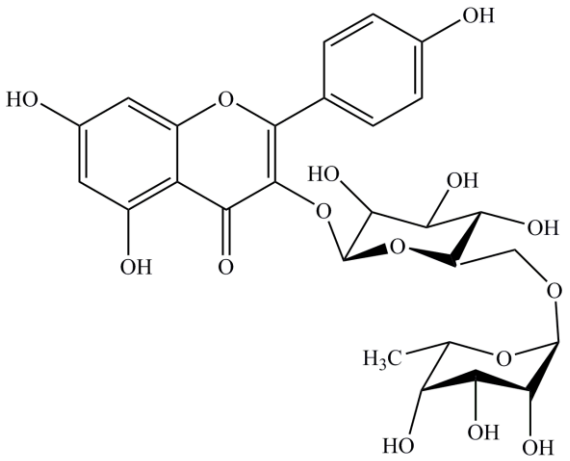
[8] 3,5DC



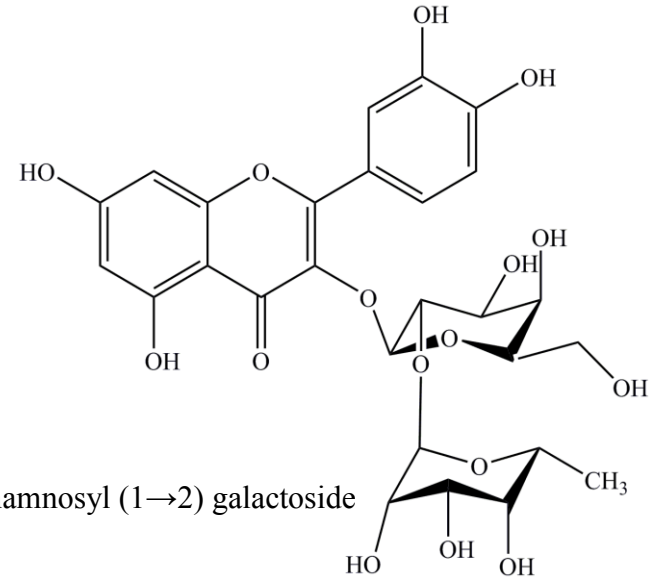
[9] 4,5DC



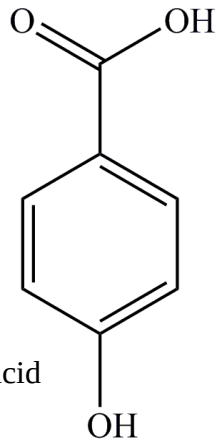
[10] Quercetin-3-*O*-rhamnosyl (1→6) glucoside



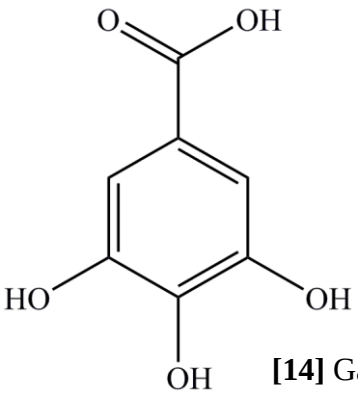
[11] Kaempferol-3-*O*-rhamnosyl (1→6) glucoside



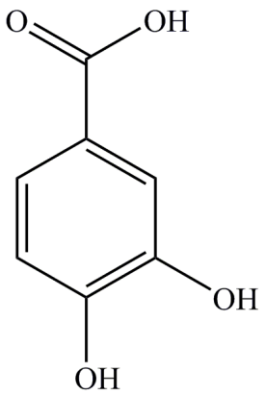
[12] Quercetin-3-*O*-rhamnosyl (1→2) galactoside



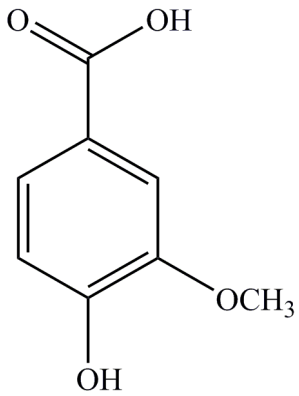
[13] *p*-hydroxybenzoic acid



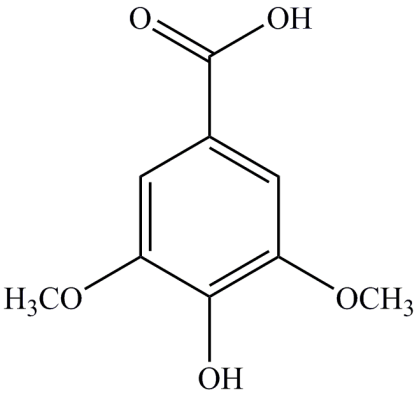
[14] Gallic acid



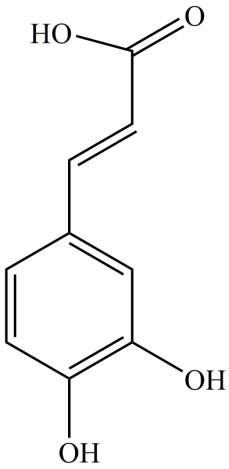
[15] Protocatechuic acid



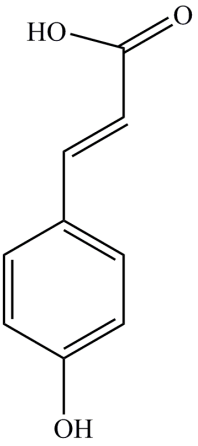
[16] Vanillic acid



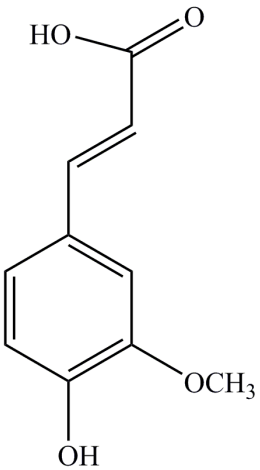
[17] Syringic acid



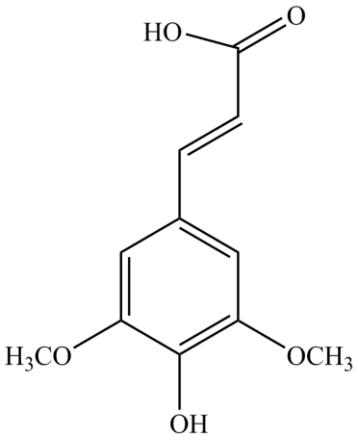
[18] Caffeic acid



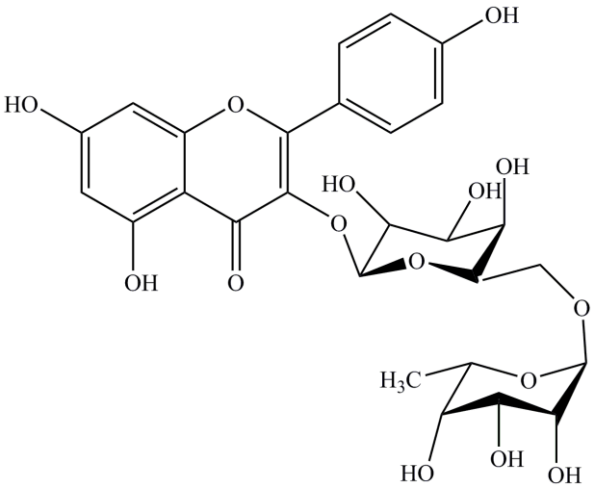
[19] *p*-coumaric acid



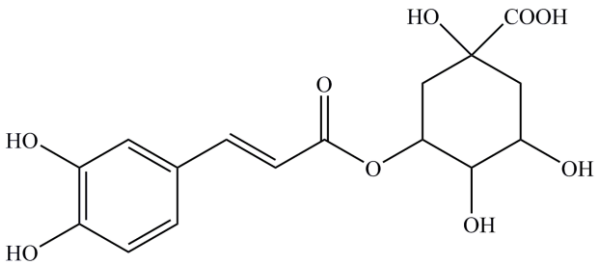
[20] Ferulic acid



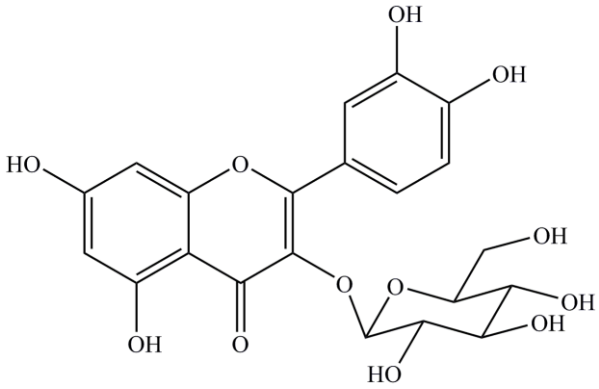
[21] Sinapic acid



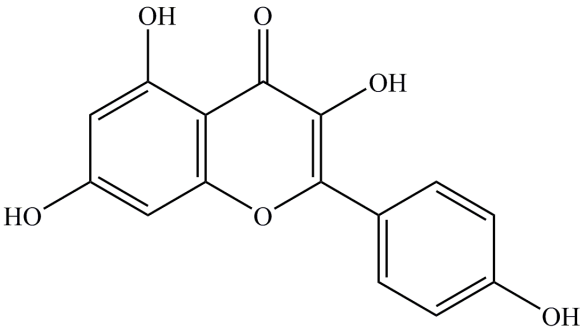
[22] Kaempferyl-3-*O*-α-L-rhamnosyl(1→6)-β-D-galactopyranoside



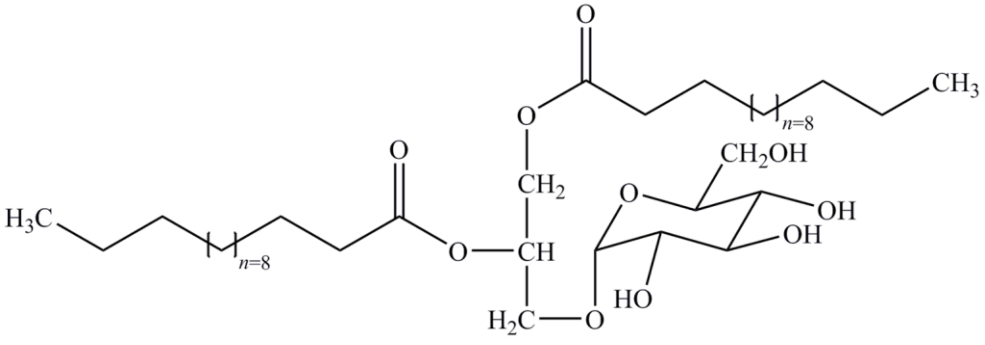
[23] CA



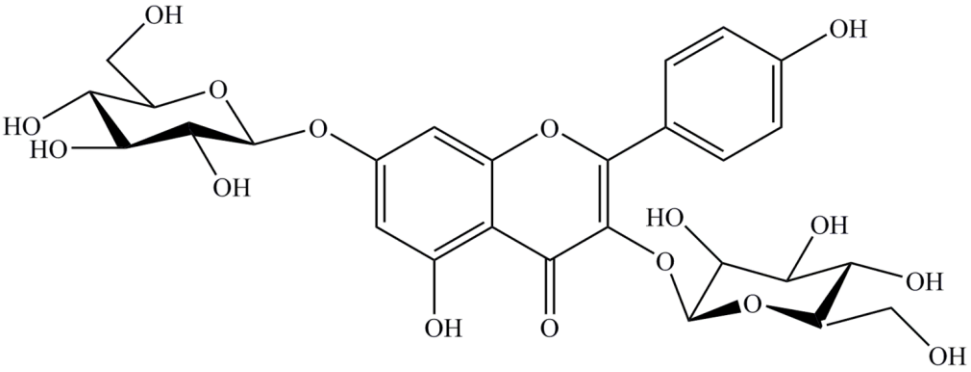
[24] Quercetin-3-*O*-β-D-glucopyranoside



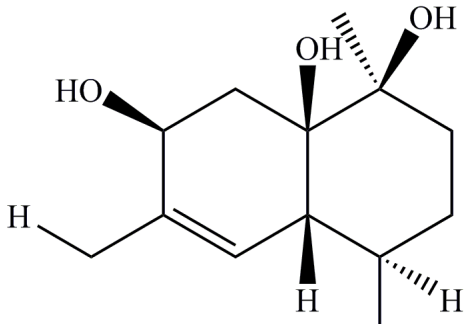
[25] Kaempferol



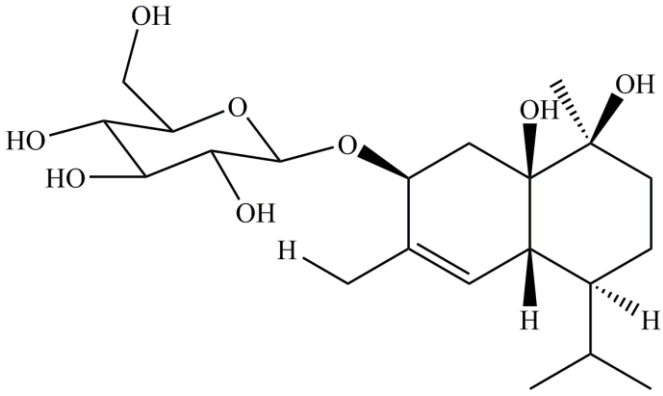
[26] 1,2-bis-dodecanoyl-3-α-D-glucopyranosyl-sn-glycerol



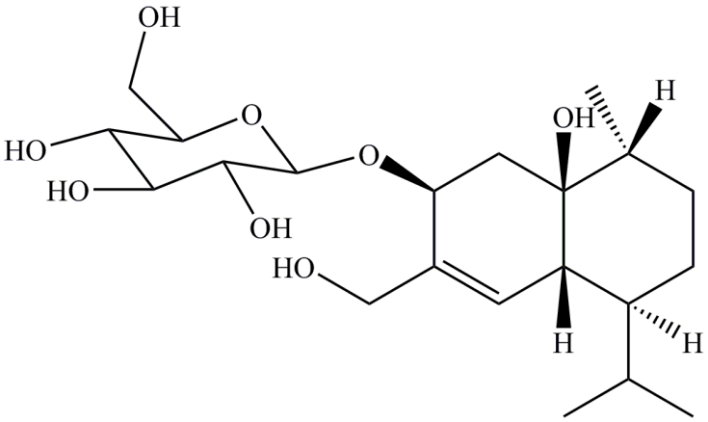
[27] Kaempferol-3,7-di-*O*-*β*-D-glucoside



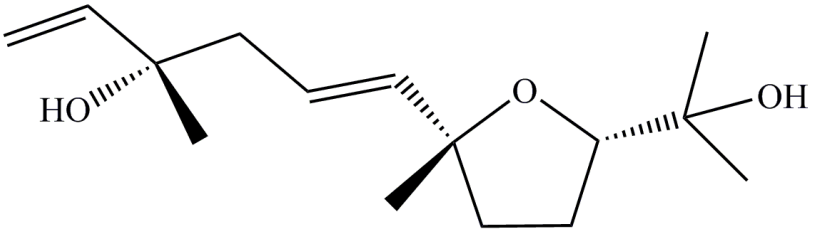
[28] Muurol-4-ene-1*β*, 3*β*, 10*β*-triol



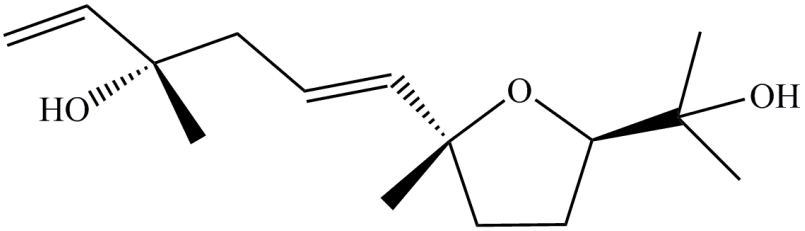
[29] Muurol-4-ene-1*β*,3*β*,10*β*-triol 3-*O*-*β*-D-glucopyranoside



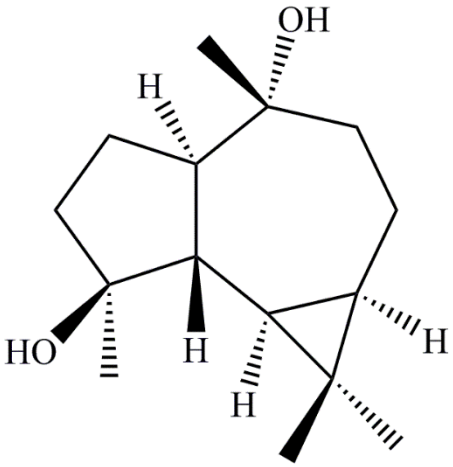
[30] Muurol-4-ene-1β,3β,15-triol 3-*O*-β-D-glucopyranoside



[31] Schensianol A



[32] Negunfurol



[33] 4β,10α-aromadendranediol

2.2.3 Pharmacological activities of *Gynura procumbens* (Lour.) Merr.

2.2.3(a) Wound healing activity

Zahra et al. (2011) reported that ethanolic extract of *G. procumbens* leaves significantly healed the wound (2 cm in diameter) created on the dorsal neck of rats. After 14 days, wounds treated by ethanol extract and intrasite gel showed signs of wound healing with less scars and healed earlier than those treated with vehicle (gum acacia) (Zahra et al., 2011).

2.2.3(b) Anticancer activity

In 2012, Nisa and the team conducted anticancer study of *G. procumbens* ethanolic extract in rats model of liver cancer induced using 7,12-dimethylbenz(*a*) antracene. Histopathology results revealed that *G. procumbens* ethanolic extract (300 mg/kg) significantly decreased proliferation of liver cells in cancer induced rats compared to untreated group, thus *G. procumbens* can be used as chemopreventive agent to inhibit carcinogenesis (Nisa et al., 2012).

2.2.3(c) Antiulcerogenic activity

G. procumbens ethanolic leaf extract was investigated for its antiulcerogenic activity at dose range of 50-400 mg/kg in absolute ethanol induced gastric lesions in rats. The animals treated with plant extracts exhibited significant ulcer protection evidenced by reduction in ulcer area and edema compared to untreated ulcer control group (Mahmood et al., 2010).

2.2.3(d) Cardiovascular activity

Kaur et al. (2012) reported cardiovascular activity of ethanolic (95 %, 75 %, 50 %, 25 %, v/v) and aqueous extracts of *G. procumbens* using rat aorta rings procedure. Aqueous extract was found to exhibit effective dose dependent vasorelaxation and negative chronotropic and ionotropic effects. Data suggested that cardiovascular activity possessed by aqueous extract can possibly be attributed to the high content of polyphenolic compounds (Kaur et al., 2012).

2.2.3(e) Ultraviolet (UV) protective activity

Protective activity of *G. procumbens* on photoaging skin caused by UV radiation has been evaluated by Kim and the team in year 2011 using human dermal fibroblasts. The result showed that 20 μg/mL of ethanolic extract of *G. procumbens* inhibited matrix metalloproteinase-1 (MMP-1) and MMP-9 expressions up to 70 % and 73 %, respectively. The extract effectively reduced ROS production and showed marked inhibitory effect on pro-inflammatory cytokine mediators, interleukin-6 (IL-6) and IL-8 in human HaCat keratinocyte (Kim et al., 2011).

2.2.3(f) Immunomodulatory activity

Effects of *G. procumbens* leaves ethanolic extract on immunocompetent T cells (CD4+ T cells, CD4+CD25+ T cells, and B220+ cells) were investigated by Dwijayanti and Rifa'I to study its immunomodulatory activity in splenic cells. The result indicated that *G. procumbens* ethanolic extract increased the production of T cells compared to control. The study concluded that *G. procumbens* possessed immunomodulatory activity

and only a very minimal dose (0.1 μg/mL and 1.0 μg/mL) was required to promote T cell activation (Dwijayanti & Rifa'i, 2015).

2.2.3(g) Antihypertensive activity

Water extract (300 and 600 mg/kg) of *G. procumbens*, orally fed via gastric gavage to spontaneously hypertensive rats for four weeks showed significant lowering of mean atrial pressure and heart rate in dose dependent manner. Significant increase in urine flow rate was also observed in treated spontaneously hypertensive rats, hence removed excess sodium and water. *G. procumbens* water extract also showed antihypertensive activity by inhibiting pressor responses induced by acetylcholine, phenylephrine, methoxamine, angiotensin II, and isoprenaline. Overall, the extract was able to lower blood pressure through non selective pathway by stimulating vasodilation, heart stabilization and diuretic effect (Kaur et al., 2013).

2.2.3(h) Anti-inflammatory activity

Water and ethyl acetate fractions of the ethanolic extract of *G. procumbens* were subjected to anti-inflammatory evaluation against ear inflammation induced by croton oil. The water fraction did not show any anti-inflammatory effect, while ethyl acetate fraction showed good anti-inflammatory activity by inhibiting increase in ear thickness due to inflammation. Among its sub-fractions, hexane (inhibition 44.6 %) and toluene (inhibition 34.8 %) sub-fractions showed comparable activity to reference drug, hydrocortisone (inhibition 35.0 %) (Iskander et al., 2002).

2.2.3(i) Antiherpetic activity

Jarikasem and team worked on antiherpetic effects of *G. procumbens* against HSV-1 and HSV-2. The ethanolic extract of the plant showed virucidal and antireplicative activity thus it was further fractionated to afford four fractions: F1 (water), F2 (50 % MeOH), F3 (MeOH) and F4 (ethyl acetate). All fractions except F1 showed antiherpetic activity against HSV-1 and HSV-2. The active fractions contained a mixture of di-caffeoylquinic acids, mixture of β-sitosterol and stigmasterol, mixture of β-sitosteryl and stigmasteryl glucosides and 1,2-bis-dodecanoyl-3-α-D-glucopyranosyl-*sn*-glycerol, all showed potent antiherpatic activity against HSV-1 and HSV-2 (Jarikasem et al., 2013).

2.2.3(j) Antidiabetic activity

The leaves of *G. procumbens* were subjected to sequential extraction in aqueous ethanol of various percentages (95 %, 75 %, 50 %, 25 %) and tested for antidiabetic activity in streptozotocin-induced diabetic rats. In acute study, the extracts lowered fasting blood glucose level significantly in diabetic rats; however, the lowering effect was the greatest in 25 % ethanolic extract. Furthermore, in sub-chronic study, the 25 % ethanolic extract exerted highest fasting blood glucose lowering effect by 49.38 % and 65.43 % on day 7 and 14 respectively, similar to effect of metformin (7^{th} day: 46.26 % and 14^{th} day: 65.42 %). In addition, 25 % ethanolic and aqueous extracts suppressed peak fasting blood glucose in subcutaneous glucose tolerance test for 90 min, which was comparable to the effect exerted by metformin (Algariri et al., 2013).