ISOLATION AND IDENTIFICATION OF CHEMICAL MARKERS FROM *LABISIA PUMILA* (BLUME) AND PREPARATION OF STANDARDISED AND NANOFORMULATED EXTRACTS FOR ANTI-OBESITY, ANTI-UTERINE FIBROID AND ANTI-CERVICAL CANCER STUDIES

by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

APPENDICES

LIST OF ABBREVIATIONS

A	Pre-exponential factor
AAS	Atomic absorption spectroscopy
ACN	Acetonitrile
ALT	Alanine transaminase
API	Active pharmaceutical ingredients
ARASC	Animal Research and Service Centre
AS	Arsenic
AST	Aspartate transaminase
AUC	Area under the curve
BMI	Body mass index
CA	Caffeic acid
Cd	Cadmium
CH_3COOH	Acetic acid
C_{max}	Peak concentration
CL	Clearance
COMT	Catechol-O-methyl transferase
CO_2	Carbon dioxide
CV	Coefficient of variation
DEPTQ	Distortionless enhancement by polarisation transfer with retention of quaternaries
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DOX	Doxorubicin
DTBP	2,4-di-tert-butylphenol
Ea	Activation energy
EGCG	Epigallocatechin gallate
ESI	Electrospray ionisation
EtOH	Ethanol
ETP	Etoposide
et al	Else where or and other

FBS	Fetal bovine serum
FRIM	Forest Research Institute of Malaysia
FTIR	Fourier Transform Infrared
FPP	Finished pharmaceutical products
GA	Gallic acid
GAVI	Global Alliance for Vaccines and Immunization
GC	Gas chromatography
GC-MS	Gas chromatography-mass spectrometry
GIT	Gastrointestinal tract
H_2O	Water
HCl	Hydrochloric acid
HCT-116	Human colorectal carcinoma cell line
HDL-C	High density lipoprotein-cholesterol
HFD	High-fat diet
Hg	Mercury
HIFBS	Heat-inactivated fetal bovine serum
HMG-CoA	3-hydroxy-3-methylglutaryl coenzyme A
HPLC	High performance liquid chromatography
HPV	Human papillomavirus
HV	High voltage
IARC	International Agency for Research on Cancer
IC_{50}	Half maximal inhibitory concentration
ICH	International Conference on Harmonisation
IL-1β	Interleukin-1beta
IL-6	Interleukin-6
i.v.	Intravenous
KBG	Kue-chin-fu-ling-man
KBr	Potassium bromide
KCL	Potassium chloride
K_e	Elimination rate constant
L. pumila	*Labisia pumila*
LA	Labisiaquinone A
LC-MS	Liquid chromatography-mass spectrometry

LDH	Lactate dehydrogenase
LDL-C	Low density lipoprotein-cholesterol
LLP	Liposome of *Labisia pumila* standardised extract
ln C	Natural logarithm of concentration
ln k	Natural logarithm of rate constant
LOD	Limit of detection
Log k	Logarithm of rate constant
LOQ	Limit of quantification
LPE	*Labisia pumila* standardised extract
LPS	Lipopolysaccharide
MCF-7	Human breast adenocarcinoma cell line
MDA	Malondialdehyde
MDI	Mixture of methyl isobutyl xanthine, dexamethasone, and insulin
MeOD	Deuterated methanol
MeOH	Methanol
MLT	Microbial limit test
MOMC	Mixture of the 5 selected marker compounds
MRSA	Methicillin-resistant *Staphylococcus aureus*
MS	Mass spectrometry
MSA	Mannitol salt agar
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
n	Number of samples
N_2	Nitrogen
NaCl	Sodium chloride
NF-kB	Nuclear factor kappa B
NIH	National Institute of Health
NMR	Nuclear magnetic resonance
NOAEL	No-observed-adverse-effect level
OECD	Organisation for Economic Cooperation and Development
OD	Optical density
Orl	Orlistat
Pb	Lead
PBS	Phosphate-buffered saline

PC3	Human prostate cancer cell line
PCA	Principal component analysis
PCS	Photon correlation spectroscopy
PDI	Poly dispersity index
PGE_2	Prostaglandin E_2
PMA	phorbol 12-myristate 13-acetate
p-NPB	p-nitrophenyl butyrate
PPL	Porcine pancreatic lipase
Psi	Pound per square inch
PTFE	Polytetrafluoroethylene
R	Rutin
R^2	Coefficient of determination
RH	Relative humidity
ROS	Reactive oxygen species
RPMI-1640	Roswell Park Memorial Institute medium
RVSEB	Rappaport Vassiliadis *Salmonella* enrichment broth
SCDA	Soybean Casein Digest Agar
SDA	Sabouraud Dextrose Agar
SEM	Standard error of mean
SK-UT-1	Uterine fibroid cell line
SPSS	Statistical Package for the Social Sciences
Sur	Suramin
$t_{1/2}$	Biological half life
TC	Total cholesterol
TEM	Transmission electron microscopy
TG	Triglycerides
TLC	Thin layer chromatography
T_{max}	Time to reach peak concentration
TNF-α	Tumor necrosis factor-alpha
UK	United Kingdom
UPLC	Ultra performance liquid chromatography
USA	United States of America
USP	United States Pharmacopoeia

UTI	Urinary tract infection
UV-Vis	Ultra violet-visible
v/v	Volume to volume
v/wt	Volume to weight
V_d	Volume of distribution
VEGF	Vascular endothelial growth factor
Vpp	Voltage peak-to-peak
WHO	World Health Organisation
XLD	Xylose-lysine-deoxycholate
Zen	Zenoctil
Zp	Zeta potential
ZS	Zetasizer
1/C	Inverse of concentration
1/T	Inverse of temperature
^{1}H-NMR	Proton nuclear magnetic resonance
2D-COSY	Two dimensional correlation spectroscopy
2D-HMBC	Two dimensional heteronuclear multiple-bond correlation
2D-HSQC	Two dimensional heteronuclear single quantum correlation
2D-NMR	Two dimensional nuclear magnetic resonance
2D-NOESY	Two dimensional nuclear overhauser effect spectroscopy
3T3-L1	Adipocyte cell line
4,5-DCQA	4,5-di-caffeoylquinic acid
5-FU	5-fluorouracil
^{13}C-NMR	Carbon-13 nuclear magnetic resonance

LIST OF UNITS

cfu	Colony forming unit
cm	Centimetre
cm^{-1}	Per centimetre
g	Gram
g/kg	Gram per kilogram
h	Hour
hrs	Hours
Hz	Hertz
IU	International unit
k	Rate constant
K	Kelvin
kg	Kilogram
kJ	Kilojoule
kV	Kilovolt
L	Litre
M	Molar
m/z	Mass-to-charge ratio
µg	Microgram
mg	Milligram
mg/dL	Milligram per decilitre
µg/mL	Microgram per millilitre
MHz	Megahertz
µL	Microlitre
mL	Millilitre
µm	Micrometre
µM	Micro mole
mm	Millimetre
mmol/L	Millimole per litre
mol	Mole
nm	Nano metre
ppm	Parts per million

LIST OF SYMBOLS

α	Alpha
β	Beta
ºC	Degree Celsius
δ	Delta
=	Equal
>	Greater than
≥	Greater than or equal to
λ	Lambda
<	Less than
≤	Less than or equal to
_	Minus
x	Multiplication
/	Per
%	Percentage
π	Pi
+	Plus
±	Plus minus
θ	Theta
ζ	Zeta potential

PEMENCILAN DAN PENGENALPASTIAN PENANDA KIMIA DARIPADA *LABISIA PUMILA* (BLUME) DAN PENYEDIAAN EKSTRAK TERPIAWAI DAN TERFORMULASI NANO UNTUK KAJIAN ANTI-OBESITI, ANTI-FIBROID UTERUS DAN ANTI-KANSER SERVIKS

ABSTRAK

Labisia pumila Blume, dikenali setempat sebagai Kacip Fatimah, telah lama digunakan sebagai tonik wanita dan produk kesihatan. *L. pumila* juga telah digunakan secara tradisional untuk merawat disentri, dismenorea dan gonorea. Kajian ini bertujuan untuk memencilkan dan mengenal pasti penanda kimia yang berkaitan dengan anti-obesiti, anti-fibroid uterus dan anti-kanser serviks dari ekstrak terpiawai *L. pumila* (LPE), untuk memformulasi dan mencirikan liposom ekstrak terpiawai *L. pumila* (LLP) dan menjalankan kajian kestabilan dipercepat, toksisiti dan farmakokinetik ke atas LPE dan LLP. Penilaian aktiviti anti-obesiti secara *in vitro* dan *in vivo* serta anti-fibroid uterus dan anti-kanser serviks secara *in vitro* telah dicuba. 2,4-di-tert-butilfenol dan labisiakuinon-A telah diasingkan dan dikenal pasti menggunakan kaedah NMR, LC-MS/GC-MS, dan FTIR. Pemiawaian LPE melibatkan pemprofilan kimia dan analisis kuantitatif menggunakan 5 sebatian penanda; asid galik (GA), asid kafeik (CA), rutin (R), 2,4-di-tert-butilfenol (DTBP) dan labisiakuinon-A (LA) menggunakan kaedah HPLC yang disahkan. Jumlah sebatian penanda dalam LPE ditemui sebanyak 1.03 - 46.36 µg/mg. LPE telah berjaya diformulasi sebagai liposom (fomulasi nano) dan dicirikan menggunakan penguji saiz zeta, zeta berpotensi, TEM dan FTIR. Saiz zarah purata adalah 171.20 ± 1.53 nm dengan zeta berpotensi -44.10 ± 1.37, keputusan FTIR menunjukkan

perbezaan ciri-ciri berkaitan dengan kumpulan berfungsi. Dalam kajian kestabilan dipercepatkan, LPE dan LLP adalah lebih stabil pada suhu 25°C berbanding suhu lain yang dikaji dan jangka hayat dianggarkan (t_{90}) adalah masing-masing 19.40 dan 22.80 bulan pada 25°C. Hasil kajian anti-obesiti *in vitro* masing-masing menunjukkan perencatan aktiviti lipase pankreas LPE dan LLP sebanyak (di 100 µg/mL) 21.85 ± 1.39 dan 40.06 ± 2.56%. Orlistat (kawalan positif) pada 100 µg/mL menunjukkan perencatan yang lebih tinggi ke atas lipase pankreas *in vitro* berbanding LPE dan LLP. LPE dan LLP mempamerkan aktiviti perencatan tinggi pada adiposit 3T3-L1 masing-masing dengan 94.30 ± 0.92 and 98.20 ± 1.49%. Untuk aktiviti anti-fibroid uterus secara *in vitro*, assai MTT telah digunakan dan peratusan perencatan untuk LPE adalah 99.01 ± 3.23% dengan IC_{50} 14.24 ± 0.69 µg/mL, manakala peratusan perencatan untuk LLP adalah 97.99 ± 2.77% dengan IC_{50} 20.33 ± 1.03 µg/mL. Kawalan positif (doksorubisin, epigallokatekin galat and etoposide) mempamerkan kesan anti-proliferasi yang hampir sama dengan LPE dan LLP terhadap jalur sel fibroid uterus. Bagi assai kanser serviks *in vitro*, peratusan perencatan untuk LPE adalah 54.69 ± 0.41% dengan IC_{50} 185.00 ± 2.77 µg / mL, manakala peratusan perencatan untuk LLP adalah 58.99 ± 0.17% dengan IC_{50} 169.60 ± 2.49 µg/mL. Kawalan positif (5-fluorouracil) menunjukkan aktiviti yang lebih tinggi terhadap jalur sel kanser serviks berbanding LPE dan LLP. Untuk aktiviti anti-angiogenesis, LPE, LLP, DTBP dan LA menunjukkan aktiviti peratusan yang tinggi 75.41 ± 2.97, 77.36 ± 2.58, 80.16 ± 3.22 dan 83.54 ± 3.43, masing-masing. Dalam toksisiti dos akut dan berulang LPE dan LLP menunjukkan bahawa ekstrak selamat pada dos yang dipilih (250-5000 mg/kg) dalam tikus Sprague-Dawley. Tiada perubahan signifikan diperhatikan dalam ciri-ciri biokimia, hematologi dan histologi pada tikus yang dirawat berbanding dengan kumpulan kawalan normal.

Farmakokinetik LPE dan LLP telah dijalankan menggunakan kaedah HPLC bagi penentuan serentak sebatian penanda plasma selepas pemberian oral dan intravena (i.v.). Bioavailabiliti GA, CA, R, DTBP dan LA dalam LPE adalah 6.70 ± 0.21, 3.92 ± 0.15, 3.38 ± 0.14, 3.75 ± 0.12 dan 4.09 ± 0.15%, manakala bagi LLP masing-masing diperhatikan adalah 9.50 ± 0.29 ($P < 0.01$) , 5.38 ± 0.23 ($P < 0.05$), 4.47 ± 0.22 ($P < 0.05$), 5.54 ± 0.17 ($P < 0.01$) dan 5.61 ± 0.23% ($P < 0.01$). Keputusan ini menunjukkan bahawa bioavailabiliti sebatian penanda dalam LLP adalah bertambah baik berbanding LPE. Model tikus teraruh diet tinggi lemak selama 45 hari telah digunakan dalam assai anti-obesiti LPE dan LLP secara *in vivo*. Hasil kajian menunjukkan pengurangan signifikan dalam indeks jisim badan ($P < 0.001$), berat badan ($P < 0.001$), pengambilan makanan harian ($P < 0.001$), jumlah kolesterol (TC) ($P < 0.001$), trigliserida (TG) ($P < 0.05$), lipoprotein ketumpatan rendah (LDL-C) ($P < 0.001$), organ hepar ($P < 0.001$), dan berat tisu adipos ($P < 0.001$) dan peningkatan lipoprotein berketumpatan tinggi (HDL-C) ($P < 0.001$) berbanding dengan kumpulan kawalan negatif serta mempamerkan ciri histologi hepar normal dalam tikus yang dirawat dengan LPE 500 mg/kg dan LLP 500 mg/kg. Kawalan positif (zenoctil dan orlistat) didapati kurang atau sama berbanding LPE dan LLP. LPE dan LLP adalah selamat pada dos yang terpilih (250-5000 mg/kg), menunjukkan kesan anti-obesiti, anti-fibroid uterus dan anti-kanser serviks secara *in vitro* dan kesan anti-obesiti secara *in vivo*.

ISOLATION AND IDENTIFICATION OF CHEMICAL MARKERS FROM *LABISIA PUMILA* (BLUME) AND PREPARATION OF STANDARDISED AND NANOFORMULATED EXTRACTS FOR ANTI-OBESITY, ANTI-UTERINE FIBROID AND ANTI-CERVICAL CANCER STUDIES

ABSTRACT

Labisia pumila Blume, locally known as Kacip Fatimah, has long been used as female tonics and health products. *L. pumila* has also been used traditionally in the treatment of dysentery, dysmenorrhoea and gonorrhoea. This study was aimed to isolate and identify of chemical markers related to anti-obesity, anti-uterine fibroid and anti-cervical cancer from *L. pumila* standardised extract (LPE), to formulate and characterise a liposome of *L. pumila* standardised extract (LLP) and carry out accelerated stability, toxicity and pharmacokinetic studies on LPE and LLP. Evaluation of the anti-obesity, anti-uterine fibroid, anti-cervical cancer studies were attempted. 2,4-di-tert-butylphenol and labisiaquinone-A were isolated and identified using NMR, LC-MS/GC-MS, and FTIR methods. Standardisation of LPE involved chemical profiling and quantitative analysis of the 5 selected marker compounds; gallic acid (GA), caffeic acid (CA), rutin (R), 2,4-di-tert-butylphenol (DTBP) and labisiaquinone-A (LA) using a validated HPLC method. The amount of the selected marker compounds in LPE was found to be in the range of 1.03-46.36 µg/mg. LPE was successfully formulated as a liposome (nanoformulation) and characterised by zetasizer, zeta potential, TEM and FTIR. The average particle size was 171.20 ± 1.53 nm with zeta potential -44.10 ± 1.37, the FTIR results showed characteristic differences in related to functional groups. In accelerated stability study, LPE and

LLP were more stable at 25°C compared to other temperatures studied and the estimated shelf life (t_{90}) was 19.40 and 22.80 months, respectively, at 25°C. For the inhibition effect on pancreatic lipase activity of LPE and LLP at 100 µg/mL, the inhibition percentage was at 21.85 ± 1.39 and 40.06 ± 2.56%, respectively. Orlistat (positive control) at 100 µg/mL showed better inhibition on pancreatic lipase as compared to LPE and LLP. LPE and LLP exhibited high inhibition activity on 3T3-L1 adipocytes at 94.30 ± 0.92 and 98.20 ± 1.49%, respectively. For the anti-uterine fibroid activity, MTT assay was used and the percentage inhibition for LPE was 99.01 ± 3.23% with IC_{50} of 14.24 ± 0.69 µg/mL, while the percentage inhibition for LLP was 97.99 ± 2.77% with IC_{50} of 20.33 ± 1.03 µg/mL at 100 µg/mL. The positive controls (doxorubicin, epigallocatechin gallate and etoposide) almost showed similar anti-proliferative effect on uterine fibroid cell line as compared to LPE and LLP. For the anti-cervical cancer assay, the percentage inhibition for LPE was 54.69 ± 0.41% with IC_{50} of 185.00 ± 2.77 µg/mL, while the percentage inhibition for LLP was 58.99 ± 0.17% with IC_{50} of 169.60 ± 2.49 µg/mL at 200 µg/mL. Positive control (5-fluorouracil) possessed higher activity on cervical cancer cell line as compared to LPE and LLP. For the anti-angiogenesis activity, LPE, LLP, DTBP and LA shows high percentage activity 75.41 ± 2.97, 77.36 ± 2.58, 80.16 ± 3.22 and 83.54 ± 3.43, respectively. In the acute and repeated dose toxicity of LPE and LLP, the extracts were safe at the selected doses (250-5000 mg/kg) in Sprague-Dawley rats. Non-significant changes were observed in biochemical, haematological and histological features in treated rats compared to normal control group. The pharmacokinetics of LPE and LLP were performed using HPLC method for the simultaneous plasma determination of the selected marker compounds after oral and intravenous (i.v.) administration. The observed bioavailability of GA, CA, R, DTBP and LA in LPE

was 6.70 ± 0.21, 3.92 ± 0.15, 3.38 ± 0.14, 3.75 ± 0.12 and 4.09 ± 0.15%, while in LLP was 9.50 ± 0.29 ($P < 0.01$) , 5.38 ± 0.23 ($P < 0.05$), 4.47 ± 0.22 ($P < 0.05$), 5.54 ± 0.17 ($P < 0.01$) and 5.61 ± 0.23% ($P < 0.01$), respectively. These results indicated that the bioavailability of selected marker compounds in LLP was improved significantly compared to LPE. A 45 days high-fat diet induced rat model was used for the *in vivo* anti-obesity assay of LPE and LLP. The results of showed significant reduction in body mass index ($P < 0.001$), weight ($P < 0.001$), daily food intake ($P < 0.001$), total cholesterol (TC) ($P < 0.001$), triglycerides (TG) ($P < 0.05$), low density lipoprotein-cholesterol (LDL-C) ($P < 0.001$), liver organ ($P < 0.001$), and adipose tissue weight ($P < 0.001$) and increased high density lipoprotein-cholesterol (HDL-C) ($P < 0.001$) as compared to the negative control group and also exhibited normal histology features in the liver of the rats treated with LPE 500 mg/kg and LLP 500 mg/kg. The positive controls (zenoctil and orlistat) were found with less or similar activity as compared to LPE and LLP. The selected doses (250-5000 mg/kg) of LPE and LLP were found safe, exhibited anti-obesity, anti-uterine fibroid and anti-cervical cancer effects.

CHAPTER ONE: INTRODUCTION

1.1 Background

Plants have been used as a source of medicine since ancient times up to date. Large number of plants are used by world's population as an important source for primary health care conditions (Sahu *et al*., 2010). Recently, researchers showed high interest to search for new medicines from plant source. Many of the medicinal plants like *Panax ginseng*, *Camellia sinensis*, *Allium sativum*, *Hypericum perforatum*, *Ginkgo biloba* and *Zingiber officinale* have gained popularity for the prevention or management of wide number of diseases (Deodhar and Shinde, 2015). In German, more than 70% of the physicians prescribe herbs. Medicinal plants are generally considered to be safe and effective agents. Therefore, people every year turn to herbal medicine because they believe plant remedies are free from undesirable side effects (Nasri and Shirzad, 2013). Preparation of new drugs from medicinal plants as reliable source has also been considered. Nowadays, researchers more than before are dependent on medicinal plants for invention of new drugs with less adverse effects (Rafieian-Kopaei, 2011). Majority of the people in the developing countries still use traditional indigenous medicines as primary health care. Universally, around 85% of all medications for primary health care are originated from plants (Abbasi *et al*., 2010).

1.2 Herbs used for the treatment of obesity

Obesity is the most prevalent health problem affecting all age groups, and leads to many complications in the form of diabetes mellitus type 2, chronic heart

disease, and stroke (Hasani-Ranjbar *et al*., 2013). Many herbs are used in the traditional medicine for management of obesity such as *Zingiber officinale* and *Angelica sinensis*. Recently, pharmacological reports on the use of the medicinal plants for treatment of obesity are increasing in number. Plant extracts were used such as *Camellia sinensis, Nigella sativa* and *Irvingia gabonensi* exhibited significant reduction in body weight (Hasani-Ranjbar *et al*., 2013). Medicinal plants worth more intention and investigation as an effective treatment for the management of obesity.

1.3 Herbs used for the treatment of uterine fibroids

Uterine fibroid is a benign tumor in the uterus. Although many women with fibroids are not aware of them, the growths may cause symptoms or problems due to their size, number, or location. Herbal treatment for uterine fibroids is widely used alternatives to surgery, drug treatment or both (Liu *et al*., 2013). Many herbs were used in traditional medicine for management of uterine fibroid such as *Cinnamomum verum* and *Zingiber officinale* (Bajracharya *et al*., 2009; Van Andel *et al*., 2014). Currently, pharmacological reports on medicinal plants used for management of uterine fibroids are rapidly getting attention. Plant extracts such as *Camellia sinensis* (Zhang *et al*., 2010a), *Allium sativum* (Obochi *et al*., 2009), *Panax ginseng* (Zhu *et al*., 2015), *Cimicifuga racemosa* (Xi *et al*., 2014), *Scutellaria barbata* (Kim *et al*., 2008a) and *Euonymus alatus* (Lee *et al*., 2004) showed activity against uterine fibroids.

In addition, formulations from plants like ayurvedic formulations (Dhiman, 2014) and Nona roguy herbal formulations (Hazlina *et al*., 2005) also exhibited

activity as effective treatment for uterine fibroid. Furthermore, curcumin (Tsuiji *et al*., 2011), berberine (Tang *et al*., 2009), genistein (Di *et al*., 2008), isoliquiritigenin (Kim *et al*., 2008c), retinoic acid (Islam *et al*., 2013) and heparin (Avila *et al*., 2013) are natural compounds from natural origin that have been reported for uterine fibroid regression and symptomatic recovery.

1.4 Herbs used for the treatment of cervical cancer

Cervical cancer is a tumor arising from the cervix. Typically no symptoms are seen. Later symptoms may involve pain during sexual intercourse, pelvic pain or abnormal vaginal bleeding. Although bleeding after sex may not be serious, it could also indicate the presence of cervical cancer (Kumar *et al*., 2012). Human papillomavirus (HPV) infection founds to be responsible for the development of more than 90% of cases. Other reasons include oral contraceptives, a weak immune system and smoking. Cervical cancer usually develops from precancerous changes over 10 to 20 years. About 90% of cervical cancer cases are squamous cell carcinomas, and 10% are adenocarcinoma. Cervical cancer can be diagnosed by cervical screening followed by a biopsy. Medical imaging is then done to determine the extent of the spread of cancer (Tarney and Han, 2014).

Regarding the treatment of cervical cancer using herbs, a famous Chinese formulation called “Kung Ching Tang”, contain a mixture of eleven Chinese herbs (*Achyranthes bidentata, Angelica sinensis, Coix lacryma-jobi, Curcuma zedoaria, Cyperus rotundus, Dipsacus asper, Laminaria japonica, Prunela vulgaris, Prunus persica, Sparganium stoloniferum,* and *Vaccaria segetalis*) found effective in relieving the symptoms and reducing the tumor size (De Moura *et al*., 2002).

Furthermore, root of *Hypoxis nyasica* investigated against cervical cancer and indicated high activity (Bhanot *et al*., 2011). There is a large number of herbs yet to be investigated for treatment of cervical cancer, thus, there is high possibility for reaching a stage in the future when cervical cancer will no longer be a threat.

1.5 Problem statement

In this study, *L. pumila* a local plant was selected for studying its anti-obesity, anti-uterine fibroid and anti-cervical cancer effects. *L. pumila* is a medicinal plant grown in South East Asia. Recent scientific findings showed that *L. pumila* have the potential for different pharmacological properties such as reducing body weight in ovariectomised rats (Fazliana *et al*., 2009), but no research study was performed on anti-obesity effects on high-fat diet induced rat model.

Although a number of products manufactured from *L. pumila* are available in the market, there is still lack of information in terms of chemical components related to anti-obesity, anti-uterine fibroid and anti-cervical cancer effects of *L. pumila*. Furthermore, a new step in development of new generation of standardised herbal medicine is preparation of botanical nano-formulation to increase the solubility, stability and bioavailability of the active constituents with therapeutic activity.

1.6 Justification of research

Based on literature review, there is no research work reported on the preparation of liposome of *L. pumila* standardised extract and thereof on the toxicity of LPE and LLP. Singh *et al*. (2009), Mohd Fuad *et al*. (2007) and Mohd Fuad *et al.* (2005), reported sub-acute, teratogenicity and reproductive toxicity studies of *L.*

pumila water (H_2O) extract at 50, 1000 and 800 mg/kg, respectively without any side effect. While the present research has been designed to perform the acute toxicity and repeated dose toxicity studies of LPE and LLP. Therefore, the present research differs from reported studies in terms of parts of plant used, type of extract and method of plant extraction.

Furthermore, there is no report on stability, pharmacokinetic and bioavailability of *L. pumila* standardised extract, so, the present research has been undertaken to perform accelerated stability, pharmacokinetic and bioavailability on LPE and LLP. In addition, there is no reports on anti-obesity, anti-uterine fibroid and anti-cervical cancer effects of *L. pumila.* Therefore, anti-obesity, anti-uterine fibroid and anti-cervical cancer effects of *L. pumila* were studied. This may be helpful for herbal industries to prepare and dispense stable and effective herbal products for the treatment and management of various illnesses.

L. pumila is reported for reducing body weight in ovariectomised rats (Fazliana *et al.*, 2009), but no research study was performed on anti-obesity effects on high-fat diet (HFD) induced rat model, anti-uterine fibroid, and anti-cervical cancer. Hence, the present research was carried out to investigate the anti-obesity, anti-uterine fibroid and anti-cervical cancer effects of LPE and LLP.

1.7 Research hypothesis

The present research hypothesised that *L. pumila* standardised extract may be formulated as a nano-liposome to improve its stability and bioavailability.

Furthermore, this research hypothesised that LPE and LLP may have potential for the treatment and management of obesity, uterine fibroid, and cervical cancer.

1.8 General objective

This study generally seeks to standardise and assure the quality of *L. pumila* extract by developing new analytical techniques such as HPLC method to quantify the chemical markers in *L. pumila* extract. Furthermore, it seeks to isolate and identify the chemical markers related to anti-obesity, anti-uterine fibroid and anti-cervical cancer effects. Moreover, it seeks to prepare new formulation from standardised *L. pumila* extract using soybean phospholipids in order to improve the stability, pharmacokinetics and bioavailability of the active constituents with therapeutic activity and to determine the safety of *L. pumila* extracts thereof. In addition, it aims to demonstrate the anti-obesity, anti-uterine fibroid and anti-cervical cancer properties of standardised *L. pumila* extracts.

1.9 Research objectives

1- To isolate and identify of chemical markers from *Labisia pumila*.

2- To carry out quality control assay, standardise, optimise of extraction, prepare and characterise liposome of *Labisia pumila* extract.

3- To evaluate the stability, toxicity and pharmacokinetics of *Labisia pumila* extracts.

4- To determine the inhibition effects of *Labisia pumila* extracts on pancreatic lipase, adipocytes, uterine fibroid, cervical cancer cells and angiogenesis.

5- To determine the *in vivo* anti-obesity effect of *Labisia pumila* extracts.

1.10 Significance of present research work

Obesity is increasing at an alarming rate. In 2014 approximately 0.6 billion people were observed obese and 1.9 billion were overweight worldwide. In 2013, World Health Organisation (WHO) reported that in South-east Asia region, Malaysia has become the highest prevalence country for obesity and overweight of 14 and 42%, respectively. Uterine fibroids occur in 20-25% of the worldwide women (McDonald *et al.*, 2011). Cervical cancer is the second most commonly diagnosed cancer and third leading cause of cancer death among women in less developed countries, nearly 90% of cervical cancer deaths occurred in developing parts of the world (Torre *et al.*, 2015).

A study carried out on the effect of *L. pumila* ovariectomised rats found that the plant may break the adipocytes membrane via lipolysis process in adipocytes (Kershaw and Flier, 2004; Ayida *et al.*, 2007). Therefore, *L. pumila* extracts evaluated for the anti-obesity effect using high fat diet induced rats. It is believed that the plant contains phytoestrogen, which acts as the primary female sexual hormone. It is well known that phytoestrogens are essential to protect body against hormonal dependent cancers such as uterine and breast (Chua *et al.*, 2012). Therefore, *L. pumila* extracts evaluated for the anti-uterine and anti-cervical cancer effects using SK-UT-1(uterine fibroid cells) and HeLa cell lines, respectively. The present research introduces the potential of *L. pumila* extracts as a new herbal product for anti-obesity, anti-uterine fibroid and anti-cervical cancer offering a platform for the treatment and management to the existing worldwide obesity, uterine fibroid and cervical cancer problems.

Figure 1.1 described the flow chart of the research study. Briefly, the plant authenticated, washed and dried before optimising the extraction process which involved different extraction method, part of the plant and extracting of the plant from different locations. After that, the plant analysed for foreign matters, moisture content, total ash, acid insoluble ash, extractive values, microbial limit test and heavy metals. Then, the plant was estimated for primary and secondary metabolites and standardised by using UV-Vis, FTIR and HPLC techniques.

Later, two marker compounds were isolated and identified using UV-Vis, FTIR, NMR, LC-MS/GC-MS and HPLC techniques. For the nano-formulation of the plant extract, soybean phospholipid used to formulate the extract and the characterisation involved zetasizer, zeta potential, TEM and FTIR methods. Next, LPE and LLP were evaluated for accelerated stability study according to International Conference on Harmonization (ICH) guidelines at four storage conditions for six months.

After that, LPE and LLP evaluated for the inhibition on pancreatic lipase and 3T3-L1 adipocytes effects. Furthermore, MTT assay was used to determine the activity of LPE and LLP on SK-UT-1 and HeLa cell lines, respectively. In addition, LPE and LLP were evaluated for the oral acute and repeated dose toxicity on Sprague-Dawley rats according to OECD guidelines. For the pharmacokinetics of LPE and LLP, HPLC method was used for the simultaneous plasma determination of the selected marker compounds after oral and i.v. administration. Finally, A 45 days high-fat diet induced rat model was used for the *in vivo* anti-obesity assay of LPE

and LLP and the body mass index, weight, daily food intake, TC, TG, LDL-C, liver organ, adipose tissue weight, HDL-C and liver histology were determined.

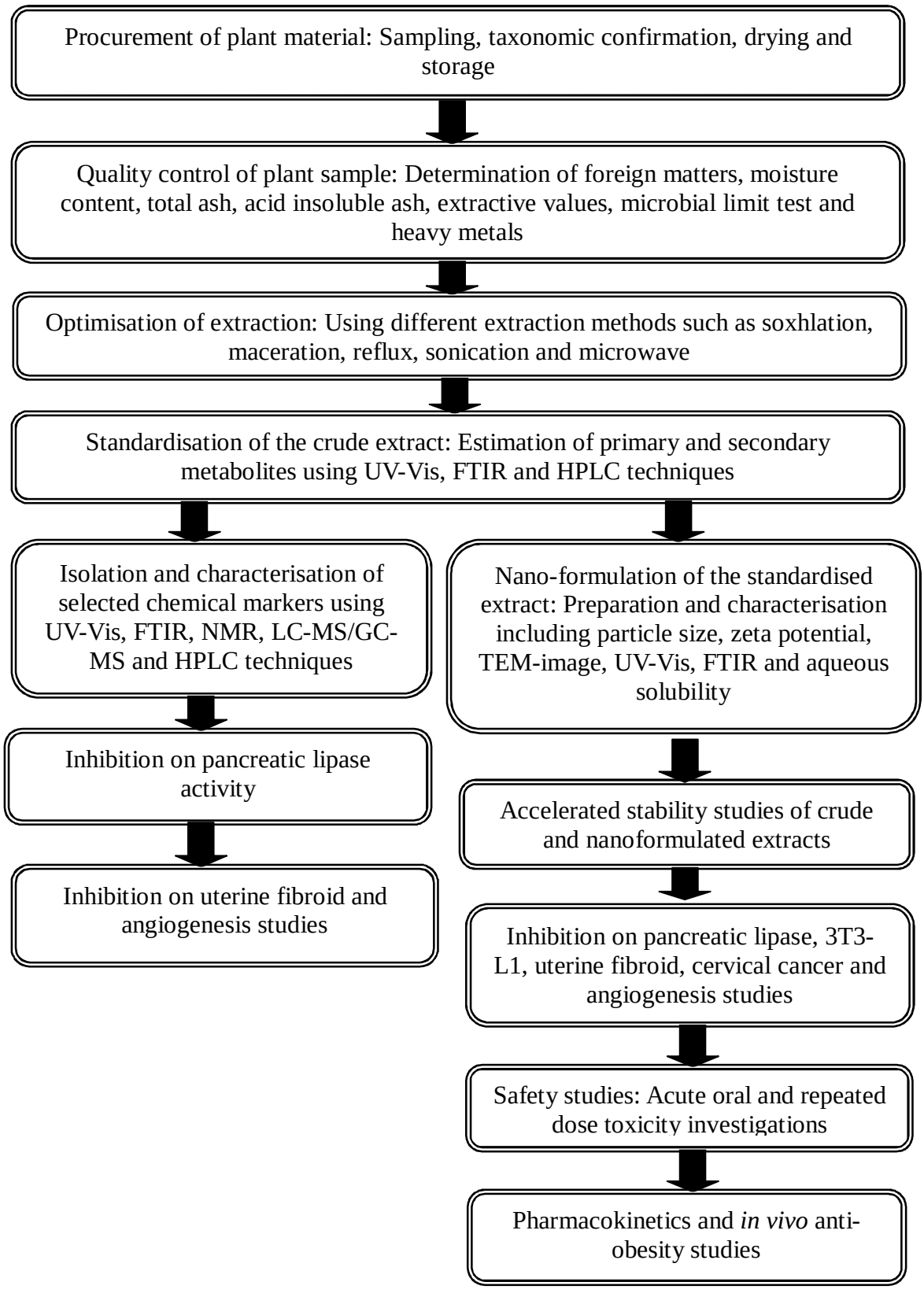


Figure 1:1: Flow chart of research study

CHAPTER TWO: LITERATURE REVIEW

2.1 *Labisia pumila*

2.1.1 Taxonomic classification

Family: Primulaceae

Genus: *Labisia*

Species: *pumila*

Scientific name: *Labisia pumila* Blume (Burkill and Haniff, 1930)

Local name: Kacip Fatimah

2.1.2 Plant description

L. pumila (synonym: *Marantodes pumilum*), is the queen of the herbs in Malaysia. It is a genus of small woody and leafy plants of the Primulaceae family that can widely be found in the tropical forest of South-East Asian countries (Chua *et al*., 2012). Figure 2.1 shows photo of *L. pumila* var. alata.

Figure 2:1: Photo of *L. pumila* var. alata (Chua *et al*., 2012)

To the natives, this plant is also known as Selusuh Fatimah, Rumput Siti Fatimah, and Akar Fatimah. Among the various varieties of *L. pumila* distributed in Malaysia, *L. pumila* var. alata, var. lanceolata and var. pumila are widely found and investigated. Differentiation of these varieties from each other can be carried out by their leaf and petiole characteristics. *L. pumila* var. pumila has a marginate petiole and ovate leaf blade shape, while var. lanceolata has a long and non-winged petiole, and var. alata has a winged petiole and red veins. The var. alata is widely used in traditional medicine preparation because it is the most commonly encountered variety in Malaysia (Sunarno, 2005; Jamal *et al*., 2003).

2.1.3 Traditional uses of *Labisia pumila*

L. pumila is traditionally used by generations of Malay women by decoction. The decoction drink is used to induce and facilitate childbirth, as well as being a post partum medication to help contract the birth channel, to regain body strength and to tone the abdominal muscles (Chua *et al*., 2012). Till now, it is taken by local people to maintain a healthy female reproductive system, to enhance sexual function, as well as to treat menstrual irregularities. Although *L. pumila* is commonly used by the female, however, in Sarawak, Malaysia the males consumed it to maintain and increase stamina (Runi, 2000). Other traditional uses of the plant include treatment for dysentery, rheumatism, gonorrhoea and excessive gas elimination from the body. It was reported that plants from the same genus (Primulaceae) also used to treat menstrual disorders and respiratory tract infections.

2.1.4 Review of chemical constituents of *Labisia pumila*

Several scientific studies on *L. pumila* described the isolation and identification of chemical compounds that contribute to the pharmacological effects. Phenolic acids and flavonoids are the most reported compounds (Chua *et al*., 2011; Karimi *et al*., 2011 and Norhaiza *et al*., 2009). Chua *et al*. (2011) reported the presence of quercetin, myricetin, kaempferol, catechin, epigallocatechin, salicylic acid, syringic acid, vanillic acid, protocatechin acid, coumaric acid, chlorogenic acid, gallic acid and caffeic acid. Ardisiacrispin A, ardisicrenoside B, ardisimamilloside H, 3-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-α-L-arabinopynansyl cyclamiretin A, irisresorcinol, belamcandol B and labisiaquinone A were reported by Avula *et al*. (2011), while 1-O-methyl-6-acetoxy-5-(pentadec-10Z-enyl) resorcinol and labisiaquinone B were identified by Al-Mekhlafi *et al*. (2012).

Fatimahol, 13,28-epoxy-oleanane glycoside and dexyloprimulanin were reported in the plant by Ali and Khan (2011). Karimi and Jaafar (2011) identified pyrogallol, naringin, daidzein, genistein, apigenin, and rutin from *L. pumila*. The presence of ascorbic acid, β-carotene and anthocyanins were reported by Norhaiza *et al*. (2009). In addition, It has been presented that 5-(pentadec-4′enyl)-resorcinol, 5-(pentadec-8′-enyl)-resorcinol and 5-(pentadec-10′-enyl)-resorcinol (irisresorcinol) as alkenyl resorcinols were found in the plant (Jamal and Houghton, 1999). Furthermore, methyl gallate was identified by Hisham *et al*. (2011). Figure 2.2 summarise the chemical structures of phytochemicals identified from *L. pumila.*

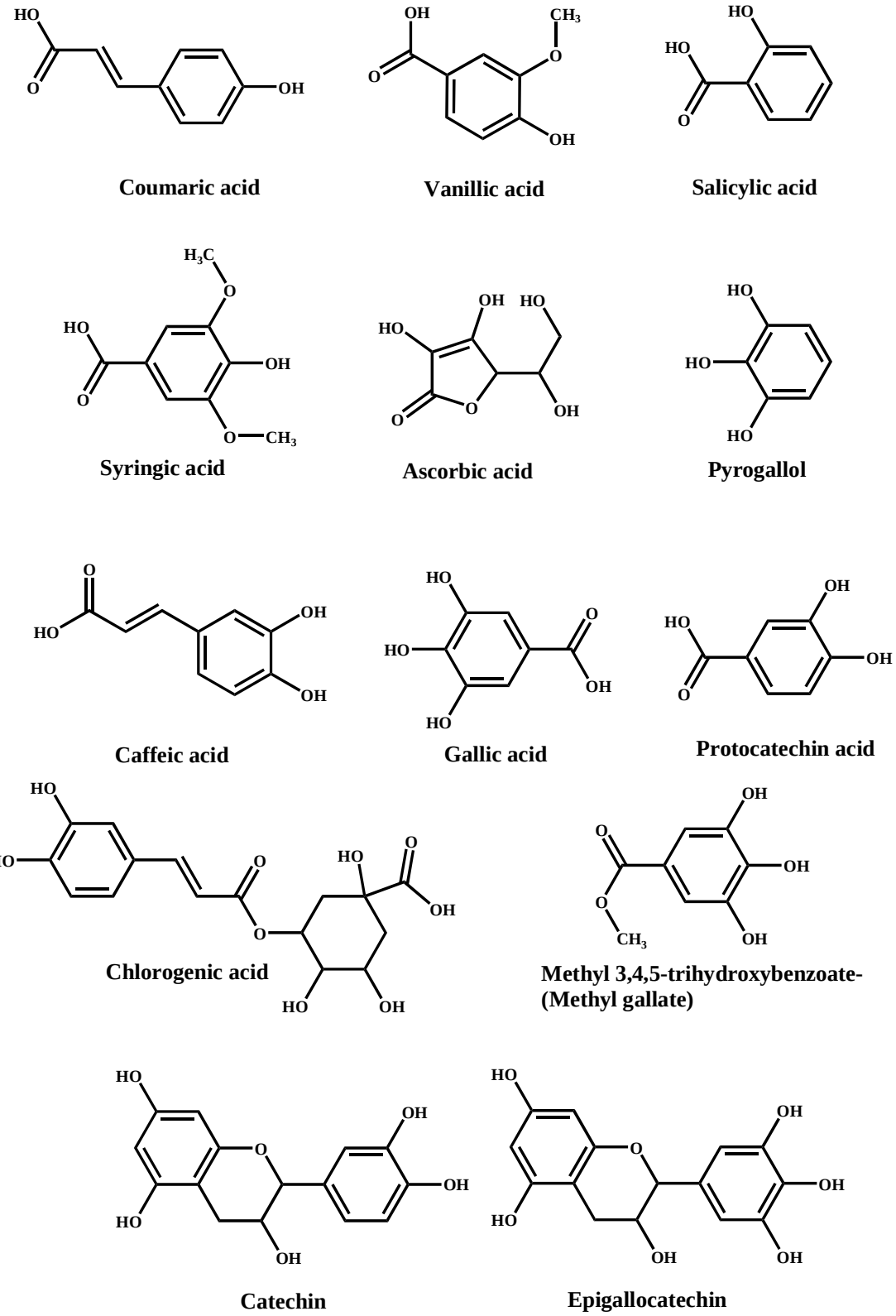
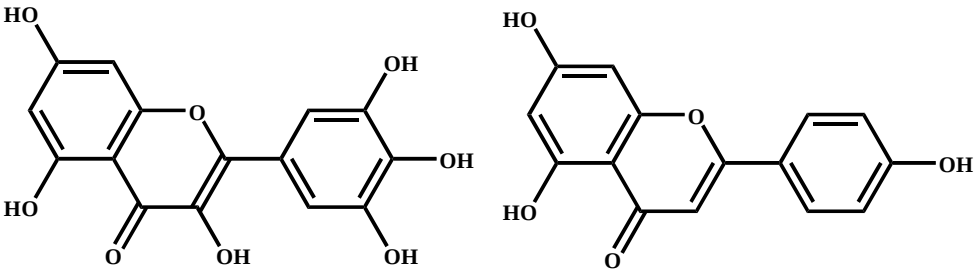
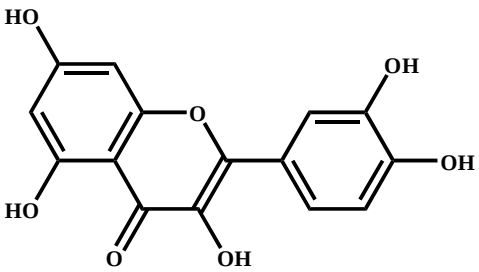


Figure 2:2: Chemical structures of phytochemicals identified from *L. pumila*

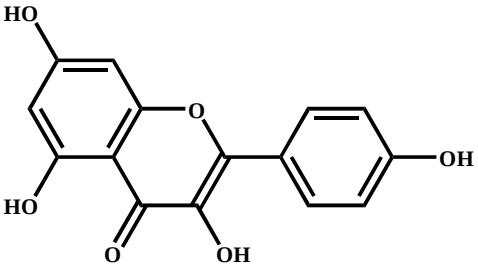


Myricetin

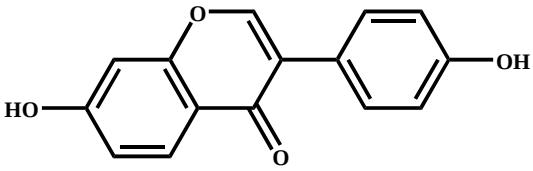
Apigenin



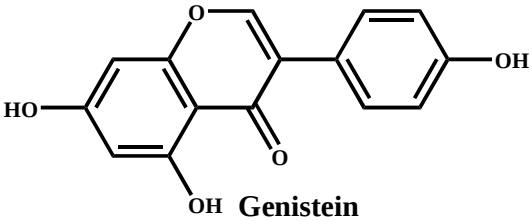
Quercetin



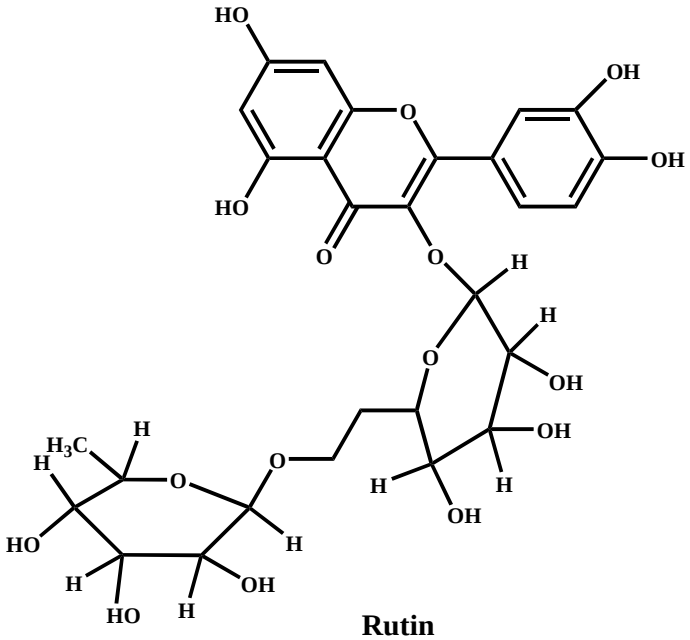
Kaempferol



Daidzein



Genistein



Rutin

Figure 2.2: Continued.

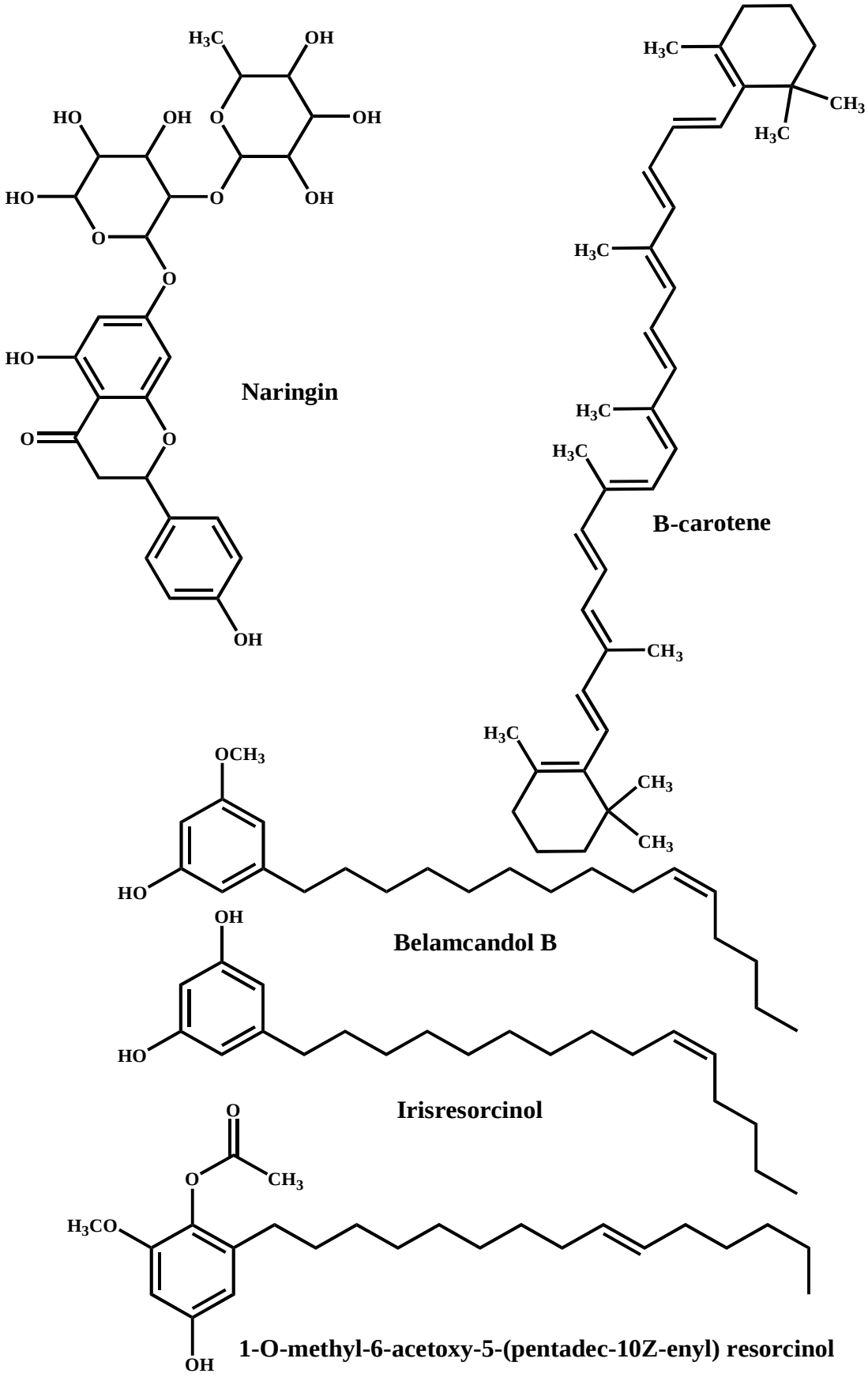
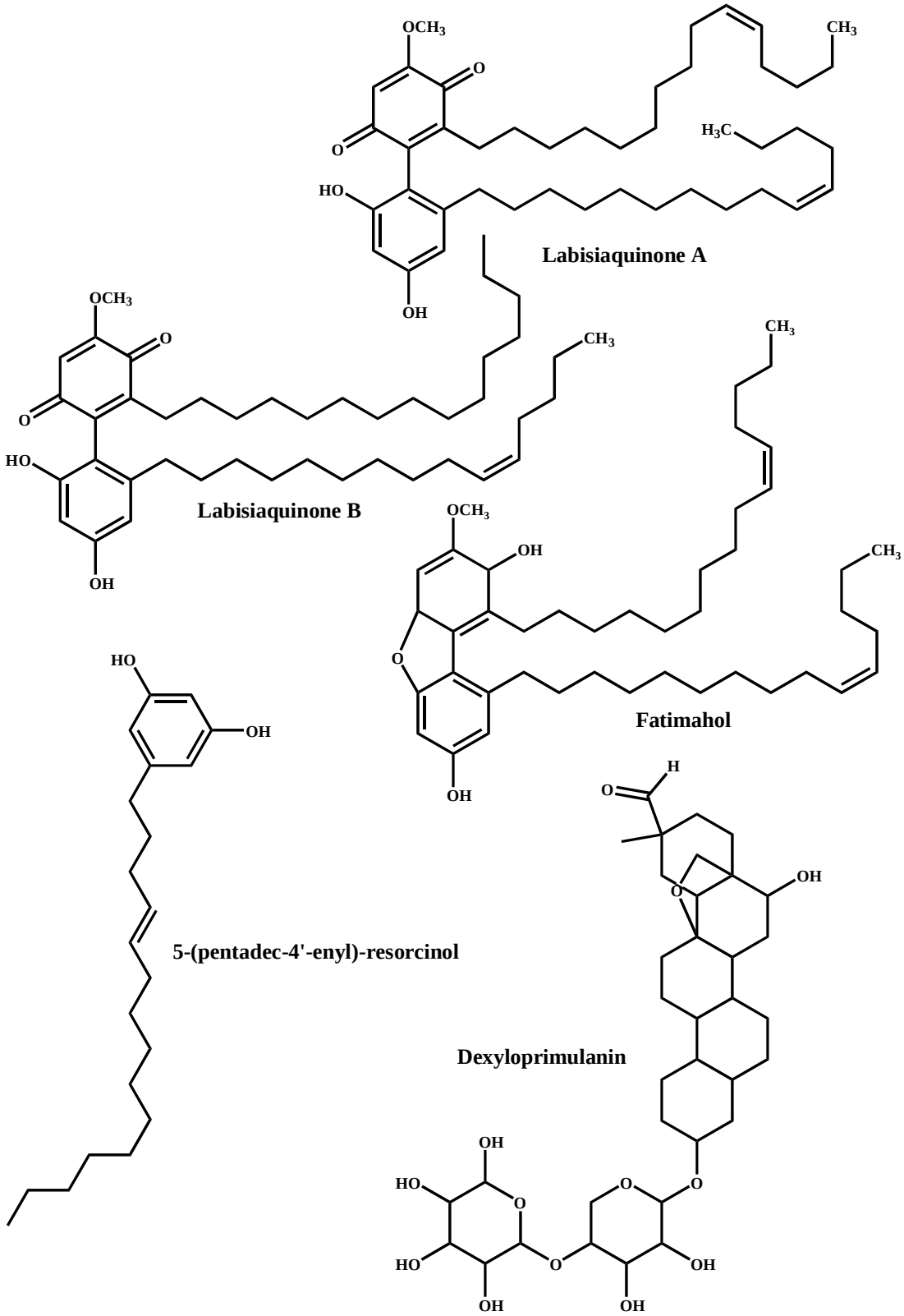


Figure 2.2: Continued



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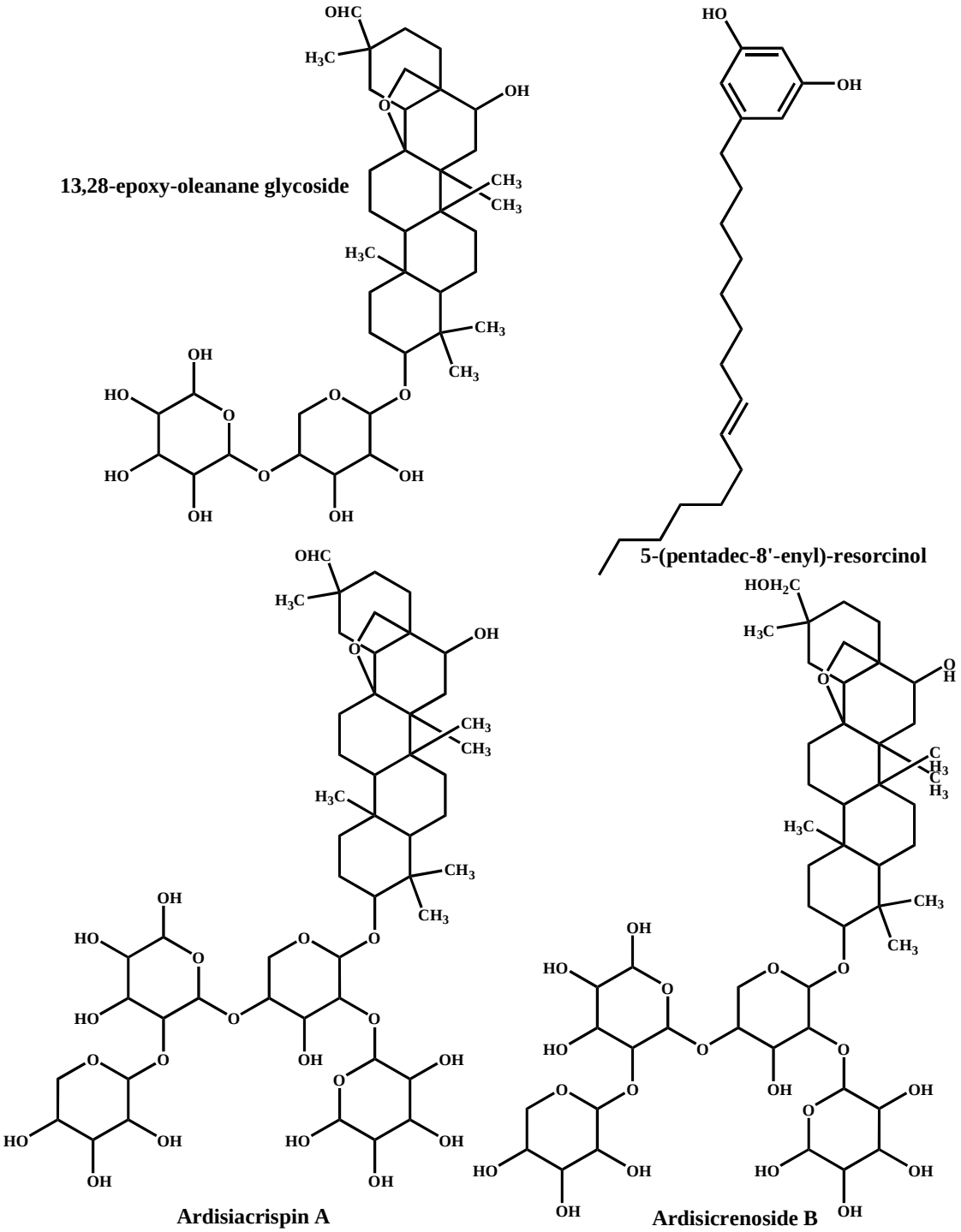

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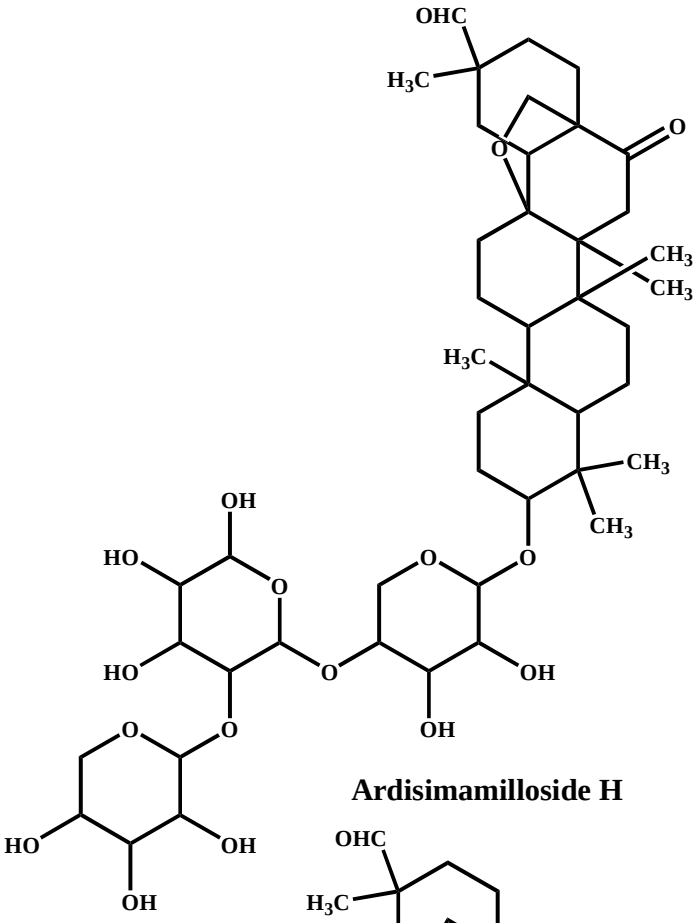
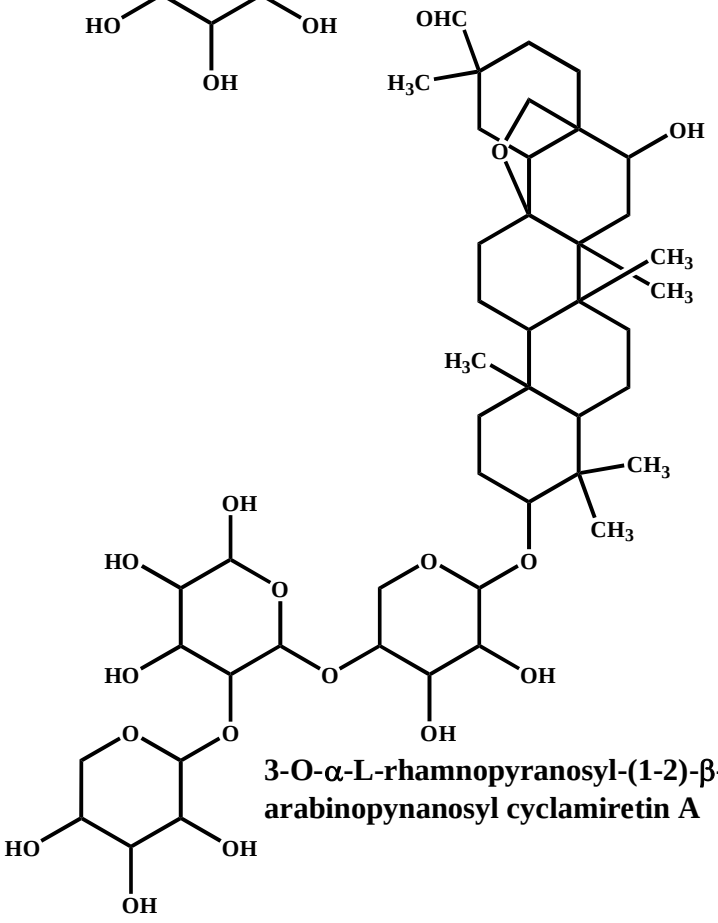


Ardisimamilloside H



3-O-α-L-rhamnopyranosyl-(1-2)-β-D-glucopyranosyl-(1-4)-α-L-arabinopynanosyl cyclamiretin A

Figure 2.2: Continued