THE EFFECT OF PHENOLIC ACTIVE FRACTION ON *Ficus deltoidea* var. *kunstleri* (KING) CORNER ON FATTY ACID-INDUCED INSULIN RESISTANCE CELL MODELS

BY

WAN NURUL ADLIN BINTI WAN YUSOF

A thesis submitted in fulfilment of the requirement for the degree of Master in Pharmaceutical Sciences (Pharmacology)

Kulliyyah of Pharmacy
International Islamic University Malaysia

APRIL 2019

ABSTRACT

Type 2 diabetes mellitus (T2DM) is the commonest type of diabetes mellitus and characterized by the insulin resistance. Many literatures showed that insulin resistance in T2DM and obesity is due to oxidative stress. Most of the traditional medicinal plants that are claimed useful in treating diabetes having antioxidant activity and believe to be beneficial in preventing the oxidative stress. The study tried to relate the relationship between antioxidant and insulin resistance. The study was conducted by determining the effect of *Ficus deltoidea* phenolics fraction that having the strongest antioxidant activity on glucose uptake of the myotubes and adipocytes in insulin resistance condition. The study consists of sequential extraction of the *F. deltoidea* and followed by fractionation using DPPH guided activity. The identified fraction by UPLC-QTOF-MS/MS that had the strongest antioxidant activity was used on insulin resistance models in order to see whether the fraction is able to enhance glucose uptake. Eleven fractions were collected. The DPPH assay result showed methanol extracts and F1 fraction (ethyl acetate fraction) was the strongest antioxidant active fraction. The identified components from the negative mode of UPLC-QTOF-MS/MS were euparin, dihydroresveratrol, feralolide, Moracin M-3'-O-β-D- glucopyranoside, cinchonain Ia, piceatannol, 3,5,4'- trihydroxystillbene, protosappanin A, cearoin, procyanidin C-1, protosappanin C, smilaxin, ceasalpins J, ceasalpins P, moracin F, protosappanin A, neosappanone A, 1-(4-hydroxybenzyl)-4-methoxy-2,7-dihydroxyphenanthrene, 1,5-Bis(4-hydroxy-3-methoxyphenyl)-1,4-pentadien-3-one, albaspidin AA, moracin O and moracin C. The identified components from the positive mode of UPLC-QTOF-MS/MS were epicatechin gallate, populnin, catechin, kaempferol, chlorogenic acid, 4-O-β-D Glucopyranosyl-trans-cinnamic acid and kaempferol-7-O-α-L-rhamnoside. For the cell culture, palmitate was able to induce insulin resistance in C2C12 myotubes but not in 3T3-L1 adipocytes. None of the strength of the fraction able to improve the insulin resistance. 10 µg/mL and 100 µg/mL of ethyl acetate fraction significantly reduced glucose uptake in C2C12 myotubes at concentration of 17.92 µM ± 2.53 and 7.40 µM ± 3.92 whereas in 3T3-L1 adipocytes, all concentration of ethyl acetate fraction reduced glucose uptake but significant at 100 µg/mL (18.6286 µM ± 1.18). Further investigation is needed to be done on the other possible mode of action by *F. deltoidea* in reconciling its anti-diabetic claims. The finding implies that not all antioxidants rich medicinal plants can reverse the insulin resistance and their beneficial effects on T2DM need detail elaboration. It is suggested that palmitate induced insulin resistance in skeletal muscle is a useful screening tool in searching a potential remedy for T2DM particularly in considering the disease pathophysiological aspect.

خلاصة البحث

يعتبر النوع الثاني من داء السُكري أكثر أنواع السُكري شيوعًا، ويتميَّز بقدرته على مقاومة الإنسولين. أظهرت العديد من الدراسات أن مقاومة الإنسولين في النوع الثاني من داء السكري والبدانة يرجِع إلى الجُهد التأكسدي. الكثير من النباتات الطبيّة التقليدية تعتبر مفيدة لعلاج السُكري لاحتوائها على أنشطة مضادة للأكسدة ونؤمن أنها مفيدة في تفادي الجهد التأكسدي. سعت هذه الدراسة إلى الربط بين مضاد الأكسدة ومقاومة الإنسولين. تم تطبيق هذه الدراسة عن طريق تحديد تأثير جزء من فينول ال *Ficus deltoidea* ، والتي لديها أقوي نشاط مضاد للأكسدة يعمل على امتصاص الجلوكوز، على النبيب العضلي والخلية الدهنيّة في حالة مقاومة الإنسولين. تتكوّن هذه الدراسة من استخراج متسلسل لل *F. deltoidea* وثُم يُتبع بتجزئته باستخدام النشاط الموجَّه بDPPH . تم استخدام الجزء المحدد عن طريق الـ UPLC-QTOF-MS/MS والذي حَمل أقوي نسبة للأنشطة المضادة للأكسدة في نماذج مقاومة للأنسولين للتأكد مما إذا كان الجزء قادرًا على تحسين امتصاص الجلوكوز. تم تجميع 11 جزئًا. أشارت نتائج فحص DPPH أن مستخرجات الميثانول و جزء ethyl acetate كانا أقوى الأجزاء النشيطة المضادة للأكسدة. المكوّنات المحددة من الوضع السلبي للUPLC-QTOF-MS/MS كانت اليوبارين، دي هيدرو ريسفيراترول، فيرالولايد، موراسين–ام–3–أو–بيتا–دي–جلوكوبيرانوسايد، سينوكوناين إي.أ، بيسيتانول، 3,5,4'ترايهيدروكسيستيلبن، بروتوسابانين أ، سيرونين، بروكيانيدين سي–1، بروتوسابانين سي، سميلاكسين، سيسالبينز جي، سيسالبينزل بي، موراسين إ، بروتوسابانين أ، نيوسابانون أ، 1-(4-هيدروكسيبينزل)–4–ميثوكسي–2، 7–ديهايدروكسيفينانثرين، 1،5–بيس(4–هايدروكسي–3–ميثوكسيفينل)– 1، 4–بينتادين–3–واحد، أباسبيدين أ.أ، موراسين أو و موراسين سي. أما المكوّنات المحددة من الوضع الإيجابي للUPLC-QTOF-MS/MS فهي إيبيكاتيكين جالات، بوبولنين، كاتيكين، كيمبفيرول، حمض الكلوروجينيك، 4–أو–بيتا–دي جلوكوبيرانوسايد– ترانس–حمض السيناميك و كيمبفيرول–7–أو–ألف–إل_رهامنوسايد. بالنسبة لثقافة الخلية، فإن الباليتيت قد استطاع أن ينتج مقاومة الإنسولين في النبيب العضلي سي2سي12، ولكن ليس في الخلية الدهنية 3تي3–إل1. م تستطع قوة الأجزاء أن تُحسِّن من مقاومة الإنسولين. 10مج/مل و 100 مج/مل من جزء أسيتيت الإيثيل قلل من نسبة امتصاص الجلوكوز في كُلٍ من النبيب العضلي سي3سي23 في تركيزة 2.53 ± 17.92 µM و ± 7.40 3.92، أما في الخلية الدهنية 3تي3–إل1، فإن كل تركيزة جُزء الأسيتيت الإيثيل قللت من نشية امتصاص الجلوكوز بنسبة (1.18 ± 18.6286 µM) 100 µg/mL.هناك احتياج لمزيدٍ من التحقيق فيما يخص طريقة الأعمال الآخرى الممكنة لل*F. deltoidea* في مصالحة إدعائاتها المضادة للسُكري. أظهرت النتائج أنه لا تستطيع كل النباتات الطبيّة الغنية بمضادات الأكسدة عكس مقاومة الإنسولين ، وأن تأثيرها المفيد على النوع الثاني من داء السُكري يحتاج إلى تفصيل دقيق. تم اقتراح ان الباليتيت يولد مقاومة الإنسولين في العضلة الهيكيليّة وهو أداة فحص مفيدة في البحث عن علاج محتمل للنوع الثاني من داء السُكري خاصة عند النظر في الجانب الفيزيولوجي للمرض.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master in Pharmaceutical Sciences (Pharmacology).

……..
Abdul Razak bin Kasmuri
Supervisor

……..
Muhammad Taher
Co-Supervisor

……..
Norazian binti Mohammad Hassan
Co-Supervisor

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master in Pharmaceutical Sciences (Pharmacology).

……..
Wan Mohd Azizi bin Wan Sulaiman
Internal Examiner

……..
Zabidah binti Ismail
External Examiner

This thesis was submitted to the Department of Basic Medical Science and is accepted as a fulfilment of the requirement for the degree of Master of Pharmaceutical Sciences (Pharmacology).

……..
Muhamad Rusdi bin Ahmad Rusmili
Head, Department of Basic Medical Sciences

This thesis was submitted to the Kulliyyah of Pharmacy and is accepted as a fulfilment of the requirement for the degree of Master in Pharmaceutical Sciences (Pharmacology).

……..
Che Suraya binti Mohd Zin
Dean, Kulliyyah of Pharmacy

DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

Wan Nurul Adlin binti Wan Yusof

Signature ..... Date .....

INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA

DECLARATION OF COPYRIGHT AND AFFIRMATION OF FAIR USE OF UNPUBLISHED RESEARCH

THE EFFECT OF PHENOLIC ACTIVE FRACTION OF *Ficus deltoidea* var. *kunstleri* (KING) CORNER ON FATTY ACID-INDUCED INSULIN RESISTANCE CELL MODELS

I declare that the copyright holders of this thesis are jointly owned by the student and IIUM.

Affirmed by Wan Nurul Adlin binti Wan Yusof

……..…….. ……..

Signature Date

ACKNOWLEDGEMENTS

In the name of Allah, The Most Gracious and The Most Merciful. All praises to Allah for giving me the strength and opportunity to embark and complete this meaningful journey. With Hiss Blessing, I finally overcome all the obstacles and tough situation with patient and finished my work as a master student.

Firstly, I want to express my ultimate gratitude for my supervisor, Associate Professor Dr. Abdul Razak Kasmuri who giving me the opportunity and never giving up on me and provide continuous support, guidance and advice. Thank you for the trust, encouragement and patient throughout my journey until the end. Also, a special thanks for my both co-supervisors, Dr Norazian bt Mohammad Hassan and Dr Taher bin Bakhtiar for all the advice, idea and guidance in completing my master study. I am also thankful the staffs in Department of Pharmaceutical Chemistry, Department of Basic Medical Sciences and Department of Pharmaceutical Technology for providing me the assistant needed.

I also dedicated my appreciation to my colleague and research mates; Nadzira, Izzati, Syima, Hanisuhana, Akilah and Aina for the endless support from the beginning until the end of my journey. Also, thanks for the members who involved directly and indirectly in the process of finishing my study.

Finally, and most importantly, a special appreciation for my beloved family, parent and husband, Fawwaz who always believe, support and pray for my success in accomplishing this journey.

TABLE OF CONTENTS

Abstract ii
Abstract in Arabic iii
Approval Page iv
Declaration vi
Copyright vii
Acknowledgement viii
List of Table xiii
List of Figures xi
List of Abbreviation xiii
List of Symbol xv

CHAPTER ONE: INTRODUCTION 1
1.1 Research Background 1
1.2 Research Problem Statement 2
1.3 Significant of Study 3
1.4 Objectives 3
1.4.1 General Objective 3
1.4.2 Specific Objective 3
1.5 Research Hypothesis 4
1.6 Study Flow 5
1.6.1 Phase 1 5
1.6.2 Phase 2 6

CHAPTER TWO: LITERATURE REVIEW 8
2.1 Diabetes Mellitus 8
2.2 Obesity, Oxidative Stress and Insulin Resistance 9
2.2.1 Relationship between Obesity, T2DM and Insulin Resistance 9
2.2.2 Physiological Insulin Signaling Pathway 11
2.2.3 Oxidative Stress, ROS and Insulin Resistance 12
2.2.4 Obesity, ROS production and Insulin Resistance 13
2.2.5 Insulin Resistance in Skeletal Muscle 16
2.2.6 Insulin Resistance in Adipocyte 17
2.3 Development of Insulin Resistance Model in Cell Lines 19
2.3.1 Palmitate causing Insulin Resistance 19
2.3.2 Insulin Resistance Cell Models 21
2.3.3 Glucose Uptake Assay 21
2.4 Antioxidants 22
2.4.1 Classification of Antioxidants 23
2.4.2 Plant antioxidants and Glycemic Control 24
2.4.3 Polyphenols and Glucose Uptake Activity at peripheral tissues 30
2.5 *Ficus deltoidea* 31
2.5.1 Phytochemicals of *F. deltoidea* 34
2.5.2 Antioxidants Activity and Antidiabetic Properties of *F. deltoidea* 34
2.5.3 LC/MS for phytochemical profiling on *F. deltoidea* 37

CHAPTER THREE: EXTRACTION AND ANTIOXIDANT ACTIVITY 40
3.1 Introduction 40
3.2 Objectives 41
3.3 Materials and Instruments 41
3.4 Methodology 42
3.4.1 Preparation of Plant Material 42
3.4.2 Sequential Extraction by Organic Solvent 42
3.4.3 Aqueous Extraction by Distilled Water 43
3.4.4 DPPH Antioxidant Assay 43
3.5 Statistical Analysis 44
3.6 Results 44
3.6.1 Percentage Yield of Extraction 44
3.6.2 DPPH assay of Crude Extracts 45
3.7 Discussion and Conclusion 45

CHAPTER FOUR: BIOASSAY-GUIDED FRACTIONATION AND ANALYSIS 49
4.1 Introduction 49
4.2 Objectives 50
4.3 Materials, Apparatus and Equipments 50
4.4 Methodology 51
4.4.1 Fractionation of Methanol Crude Extract 51
4.4.2 Antioxidant Assay 52
4.4.3 Detection and Identification of Polyphenols in Highest Antioxidant Activity Fraction 52
4.5 Statistical Analysis 53
4.6 Results 54
4.6.1 Percentage Yield of Methanol Crude Extract Fractions 54
4.6.2 Antioxidant Activity of Methanol Crude Extract Fractions 55
4.6.3 Chemical Constituent's Identification of Ethyl Acetate Fraction 56
4.7 Discussion and Conclusion 62

CHAPTER FIVE: DEVELOPMENT OF INSULIN RESISTANCE CELLS MODEL AND GLUCOSE UPTAKE ASSAY 69
5.1 Introduction 69
5.2 Objectives 71
5.3 Materials, Chemicals and Instruments 71
5.3.1 Materials 71
5.3.2 Chemicals 71
5.3.3 Equipment 72
5.4 Methodology 72
5.4.1 General Procedure for Cell Culture 72
5.4.1.1 Cell Thawing 72
5.4.1.2 Cell Sub-culturing, Maintaining and Freezing 73
5.4.1.3 Cell Counting and Seeding 73
5.4.2 Development of Insulin-resistance in 3T3-L1 Cell Line 74
5.4.2.1 Cell Growth 74
5.4.2.2 Cell Viability Test 74

5.4.2.3 Adipogenesis 75
5.4.2.4 Oil Red O Staining 75
5.4.2.5 Palmitate Induced Insulin Resistance 76
5.4.3 Development of Insulin-resistance in C2C12 Myotube Cell Line 76
5.4.3.1 Cell Growth 76
5.4.3.2 Cell Viability Test 76
5.4.3.3 C2C12 Myotube Cell Line Differentiation 77
5.4.3.4 Palmitate Induced Insulin Resistance 77
5.4.4 Glucose Uptake Assay 78
5.5 Statistical Analysis 78
5.6 Results 79
5.6.1 Morphology of 3T3-L1 Adipocytes before and after Differentiation 79
5.6.2 Quantification of Lipid Droplet in Differentiated 3T3-L1 Adipocytes after Treatment with Palmitate 79
5.6.3 Effect of Ethyl Acetate Fraction of *F. deltoidea* var. *kunstleri* on 3T3-L1 Adipocyte Viability 81
5.6.4 Morphology of C2C12 Myotubes before and after Differentiation 82
5.6.5 Morphology of C2C12 Myotubes before and after Treatment with Palmitate 82
5.6.6 Effect of Ethyl Acetate Fraction of *F. deltoidea* var. *kunstleri* Leaves on C2C12 Myotubes 83
5.6.7 Glucose Uptake Assay 84
5.6.7.1 Glucose Uptake Assay in 3T3-L1 Cell Line 84
5.6.7.2 Glucose Uptake Assay in C2C12 Cell Line 86
5.7 Discussion and Conclusion 87

CHAPTER SIX: GENERAL DISCUSSION AND CONCLUSION 95

REFERENCES 101

APPENDIX I: STANDARD CURVE 125
APPENDIX II: CHROMATOGRAM BPI PLOT 126

LIST OF TABLES

Table No.		Page No.
2.1	Herbs that showed antioxidants activity	26
2.2	The phytochemicals from the leaf extracts of different *F. deltoidea* varieties through LC/MS analysis	38
3.1	The percentage yield of *Ficus deltoidea* var. *kunstleri* leaves extract	44
3.2	IC_{50} value of different crude extracts of *Ficus deltoidea* var. *kunstleri* leaves on DPPH antioxidant activity assay	45
4.1	The gradient elution for LCMS	53
4.2	Percentage yield of crude methanol extract fraction of *Ficus deltoidea* var. *kunstleri*	54
4.3	IC50 value of methanol crude extract's fractions of *Ficus deltoidea* var. *kunstleri*	55
4.4	Components identified for negative mode using UPLC Q-TOF for ehtyl acetate fraction of *F. deltoidea* var. *kunstleri*	57
4.5	Components identified for postive mode using UPLC Q-TOF for ethyl acetate fraction of *F. deltoidea* var. *kunstleri*	60

LIST OF FIGURES

Figure No.		Page No.
2.1	Insulin signaling pathway and oxidative pathway	12
2.2	The relationship of Obesity, ROS production and Insulin Resistance	14
2.3	The Relationshi between excess adipose tissue leads to systemic inflammation and ROS production	19
2.4	The comparison of radioactive method and glucose uptake assay using varying amounts of HTCT116 colon cancer cells	22
2.5	Classification of antioxidants based on enzymatic and non-enzymatic antioxidants	24
2.6	Role of antioxidant in obesity-induces insulin resistance	25
2.7	Subgroups of polyphenols and their basic structures	29
2.8	Example of commercialize *F. deltoidea* products	32
2.9	*F. deltoidea* leaves and syconium	33
2.10	The Seven varieties of *F. deltoidea* found in Peninsula Malaysia	33
4.1	Summary of identified compounds of ethyl acetate fraction of *F. deltoidea* var. *kunstleri* in negative mode	56
4.2	The structure of identified compounds in F. deltoidea ethyl acetate fraction by LC-QTOF-MS/MS negative mode	60
4.3	Summary of identified compounds of ethyl acetate fraction of *F. deltoidea* var. *kunstleri* in positive mode	60
4.4	The structure of identified compounds in F. deltoidea ethyl acetate fraction by LC-QTOF-MS/MS positive mode	61
5.1	Morphology of 3T3-L1 cell before and after differentiation	79
5.2	Oil red O staining and quantification	80
5.3	Effects of different concentration of *F. deltoidea* var. *kunstleri* ethyl acetate fraction on 3T3-L1 viability	81
5.4	Morphology of C2C12 myotube at 4x magnification	82
5.5	Morphology of treated C2C12 myotube at 4x magnification	83

5.6 The effect of ethyl acetate fraction of *F.deltoidea* var. *kunstleri* leave on C2C12 myotubes viability 84

5.7 The effects of palmitate on 3T3-L1 adipocyte glucose uptake 85

5.8 The effect of ethyl acetate fraction of *F. deltoidea* var. *kunstleri* leaves on palmitate treated 3T3-L1 adipocyte 85

5.9 The effects of palmitate on C2C12 myotube glucose uptake 86

5.10 The effect of ethyl acetate fraction of *F. deltoidea* var. *kunstleri* on the glucose uptake of palmitate treated C2C12 myotubes 87

LIST OF ABBREVIATIONS

µg	Microgram
µL	Microlitre
µmoL	Micromolar
2-DG	2-Deoxyglucose
2-DG6P	2-Deoxyglucose-6-phosphate
4-HNE	4-hydroxy-2-nonenal
ABTS	2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)
AMPK	5’ adenosine monophosphate-activated protein kinase
ANOVA	One-way Variance Analysis
ATP	Adenosine triphosphate
BMI	Body Mass Index
BSA	Bovine Serum Albumin
BSC	Biosafety Cabinet
C	Carbon
CK-2	Ceasein kinase-2
cm	Centimeter
cm^2	Centimeter square
CO_2	Carbon Dioxide
Da	Dalton
DM	Diabetes Mellitus
DMEM	Dulbecco’s Modified Eagle Medium
DMSO	Dimethyl Sulfoxide
DPPH	1,1-diphenyl-2-picrylhydrazyl radical scavenging activity
ESI	Electrospray Ionization
et al.	and others.
F	Fraction

FBS	Fetal Bovine Serum
FFAs	Free Fatty Acids
FRAP	Ferric reducing antioxidant potential
g	gram
GACP	Good Agricultural and Collection Practices
GLUT4	Glucose Transporter Type
GS	Gas Chromatography
h	hour
HPLC	High Performance Liquid Chromatography
IBMX	3-isobutyl-1-methylxanthine
IC_{50}	Inhibition Concentration
IDF	International Diabetes Federation
Ins	Insulin
IRS-1	Insulin receptor substrate-1
JNK	c-Jun N-terminal kinase
LC-QTOF-MS/MS	Liquid Chromatography-Quadruple Time of Flight-Tandem Mass spectrometry
MAPK	Mitogen-activated protein kinase
Min	Minute
mL	Milliliter
mM	Millimolar
MS	Mass Spectrometry
MS/MS	Tandem Mass Spectrometry
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide
NADPH	Nicotinamide adenine dinucleotide phosphate
NEFAs	Non-Esterified Fatty Acids
NF-κB	Nuclear Factor kappa light chain enhancer
No	Number
NOX-4	NADPH oxidase 4

OD	Optical Density
ORAC	Oxygen Radical Absorbance Capacity
P38 MAPK	P38 mitogen-activated protein kinases
PBS	Phosphate Buffer Saline
PDA	Photo Diode Array detector
PI3-kinase	Phosphatidylinositol-4,5-bisphosphate 3-kinase
PIP_2	Phosphatidylinositol 4,5-bisphosphate
PKB/Akt	Protein Kinase B
QTOF	Quadruple Time of Flight
R	Regression
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
rpm	Revolutions per minute
Rt	Retention Time
SEA	South East Asia
SEM	Standard Error Mean
STZ	Streptozotocin
T2DM	Type 2 diabetes Mellitus
TCM	Traditional and Complementary Medicine
UPLC	Ultra-performance Liquid Chromatography Mass Spectrometry
USA	United State of America
var.	Varieties
VLC	Vacuum Liquid Chromatography
WHO	World Health Organization

LIST OF SYMBOLS

*	statistically significant denotation
±	confidence interval or error
®	Registered trademark
˚C	degree Celsius
µ	micro
P	probability
™	Trademark
α	alpha
β	beta
κ	kappa

CHAPTER ONE

INTRODUCTION

1.1 RESEARCH BACKGROUND

Diabetes mellitus is one of the epidemic diseases which affect globally. Asia is the most affected region with China and India as the top two countries where the people with diabetes (Zheng et al., 2018). About 415 million of adults have suffered from diabetes and 90% of cases are type 2 diabetes mellitus (T2DM). The International Diabetes Federation (IDF) reported about 6.8% of global mortality equivalent to 3.96 million deaths are due to diabetes mellitus in year 2010 and was raised to 5.0 million deaths in year 2015 (Zheng et al, 2018). Factors affect this rapid emerging epidemic are due to sedentary lifestyle and unhealthy diet aside from genetic predisposition. Further complication of T2DM can leads to heart disease, stroke, impaired in vision, foot ulcer and kidney damage (Jain, 2010). Among all of the complications, mortality and morbidity of the patients are mainly due to cardiovascular complication and in Asia, kidney damage in diabetes mellitus are more prevalence (Zheng et al., 2018). T2DM is a metabolic syndrome which affects the proper production of insulin and the decrease in sensitivity of cells towards the insulin stimulation. The essential precursor of the development of T2DM is insulin resistance and both T2DM and obesity linked to the development of insulin resistance especially in peripheral tissues (Al-Goblan, Alfi & Khan, 2014). Oxidative stress is one of the pathways which leads to insulin resistance in obesity and T2DM patient. Studies has discovered insulin resistance has related with obesity due to the high fat intake and glucose intake which provide extra subtract for the body to produce reactive oxygen species (ROS) and causes oxidative stress.

Oxidative stress further damage cells and signaling pathway thus leads to insulin resistance. Recently, antioxidant therapy has been introduced in preventive and management of T2DM (Bajaj, 2012). There are studies that showed antioxidant is capable as anti-diabetic especially the dietary polyphenols (Mazumder et al., 2012). Many commercial herbal medicines with antioxidant properties have anti-diabetic effects. This study is to investigate the antioxidant containing plant *Ficus deltoidea* var. *kunstleri* (King) Corner and its antioxidant active phenolic fraction on palmitate induced insulin resistance in C2C12 myotubes and 3T3-L1 adipocyte.

1.2 RESEARCH PROBLEM STATEMENT

Insulin resistance in obesity and T2DM are related to oxidative stress. High amount of free fatty acids and glucose increases ROS production and create oxidative stress due to the imbalance of the antioxidant system and increases in ROS level. Thus, external antioxidants are needed to combat the harmful condition in T2DM and obesity patients and prevent further complications. Nowadays, the commercialized herbal medicines are claimed to have anti-diabetics effect and most of the herbals have antioxidant activity. Antioxidants have several classes according to their mode of actions. Dietary polyphenols are the phytochemicals which responsible for the antioxidants activity of herbals and some of the dietary polyphenols have dual action either as antioxidant or pro-oxidants which will further deteriorate conditions if taken without consultation or proper evaluation. Some of the dietary polyphenols also have more specific mechanism in glycemic control which involve in insulin signaling pathway either inhibition or stimulation pathway. Thus, extensive investigation needs to be done on the effect of antioxidant properties of herbals which have the anti-diabetic effects. *F. deltoidea* is one of the herbal plants traditionally used as antidiabetic which contained abundant

antioxidant compounds. Other than as α-glucosidase inhibitor, this study was trying to explore other possible pathways of *F. deltoidea* as antidiabetic. *F. deltoidea* leave was used in the study to investigate the antioxidant properties of the leaves either ameliorate or deteriorate the insulin resistance condition.

1.3 SIGNIFICANT OF STUDY

The study is important to provide better understanding on the effects of antioxidant towards glycemic control and on the relationship between obesity and insulin resistance in T2DM. The preemptive claim that all antioxidants are able to cure diabetes can be explained and reconciled. The study on the antioxidant of herbs and characterizing the type of antioxidants is important to ensure their efficacy on diabetes mellitus as well. This will avoid misleading information by the consumers and the sellers. The study also illustrates the relationship between obesity causes insulin resistance in T2DM.

1.4 OBJECTIVES

1.4.1 General Objective

To evaluate the effect of the antioxidant active fractions of *F. deltoidea* extracts on insulin-resistance in C2C12 myotubes and 3T3-L1 adipocytes cell models.

1.4.2 Specific Objective

1. To investigate the most antioxidant active fraction from *F. deltoidea* extracts by bio-assay-guided fractionation using vacuum liquid chromatography.
2. To induce and develop insulin-resistant cells in C2C12 myoblast and 3T3-L1 adipocytes cell line.

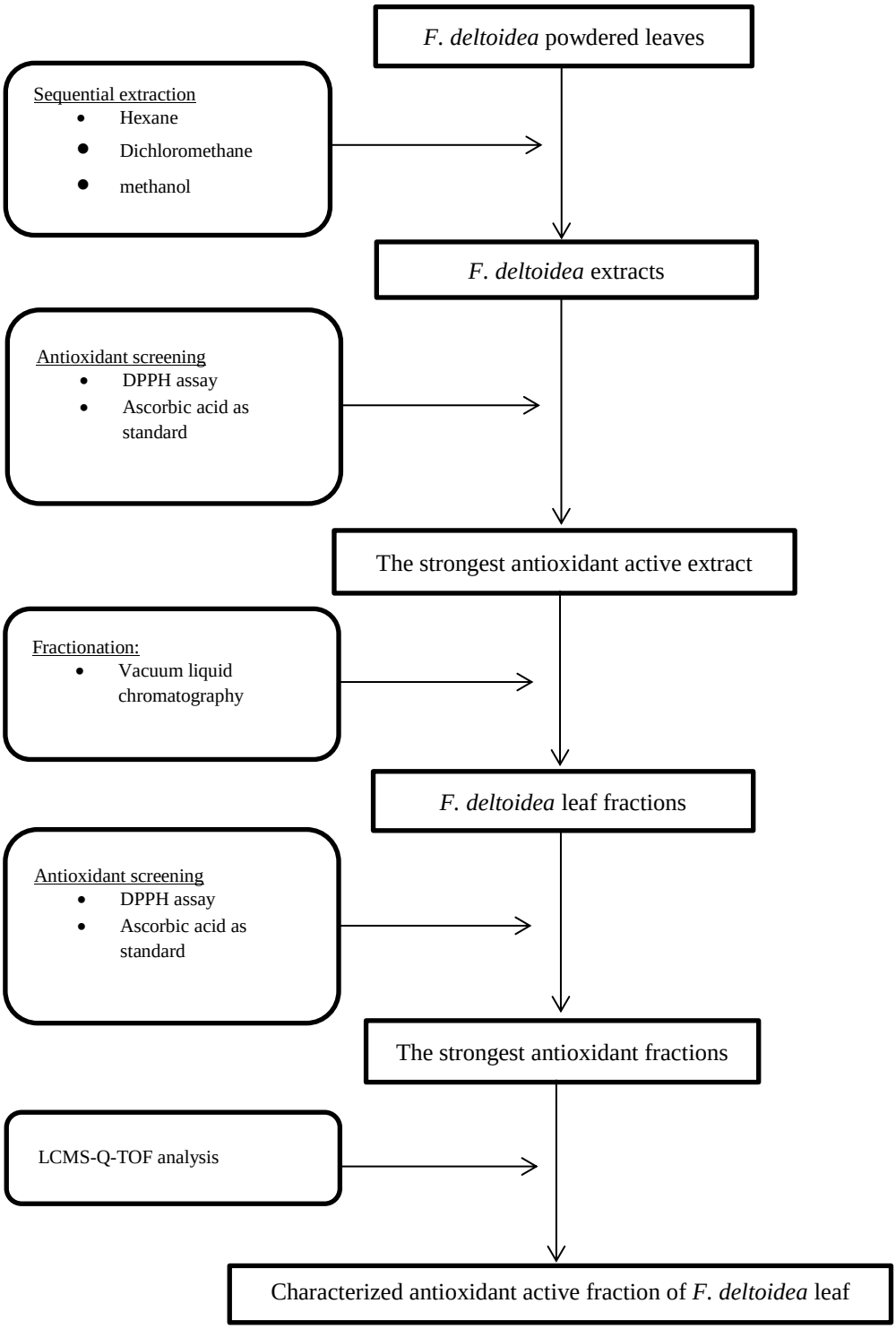
3. To evaluate the activity of the antioxidant active fraction of *F. deltoidea* leaf extract in reversing the induced-insulin resistance cell lines by glucose uptake assay.

1.5 RESEARCH HYPOTHESIS

1. Palmitate is responsible for inducing insulin resistance in C2C12 myotubes and 3T3-L1 adipocytes.
2. Phenolic compounds in *F. deltoidea* var. *kunstleri* leaves have different effects on palmitate-induced insulin resistance cell models.

1.6 STUDY FLOW

1.6.1 Phase one



1.6.2 Phase two