



PHYTOCHEMICAL INVESTIGATION OF THE STEMS
OF *TETRACERA INDICA* MERR. AND *IN-VITRO*
ANTIDIABETIC EVALUATION OF ISOLATED
COMPOUNDS

BY

MD. MAHMUDUL HASAN

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

Kulliyyah of Pharmacy
International Islamic University Malaysia

JULY, 2016

ABSTRACT

Tetracera indica Merr. (Family: Dilleniaceae) is a large, woody, rain forest climber species. It is found throughout the Malaysia and is locally and commonly known as Mempelas paya or sand paper plant. Aerial parts of *T. indica* have been traditionally used to cure different disorders including diabetes and its related infirmities in Malaysia. The traditional claims of this plant as an antidiabetic agent have not been properly scientifically evaluated. Hence, the aim of this study was to explore *in vitro* antidiabetic potential of the stems of *T. indica* as well as to isolate the compounds responsible for its *in vitro* antidiabetic property. The *in vitro* antidiabetic activity investigation of the stems ethanol extract, sub-fractions and isolated compounds from the bioactive fraction was carried out on 3T3-L1 pre-adipocytes and adipocytes. Upon thorough investigations, four flavonoids (MHQ1-Wogonin, MHQ-2-Norwogonin, MHQ-3-Quercetin, MHQ-4-Teachtchrysin) and two terpenoids (MHQ-5-Stigmasterol, MHQ-6-Betulinic acid) were isolated from the stems ethanol extract of *T. indica* and further subjected to cytotoxicity test against 3T3-L1 adipocytes with regard to evaluate their antidiabetic potential. All the compounds were isolated and purified through silica gel and sephadex LH-20 repeated column chromatography and recrystallization with different solvents. Their structures were elucidated through ¹H- and ¹³C-NMR spectroscopy. Cytotoxicity test was performed through MTT assay on 3T3-L1 pre-adipocytes to determine the safe dose of the extract, sub-fractions and all the isolated compounds for further *in vitro* antidiabetic evaluation on 3T3-L1 pre-adipocytes. The stems ethanol extract, sub-fractions and isolated pure compounds i.e. MHQ1, MHQ2, MHQ3 and MHQ4 were further subjected to adipogenesis to investigate insulin like activity or insulin sensitizing activity for the purpose of evaluating antidiabetic potential. All compounds were introduced to the cells in different safe concentrations as well as in different adipogenic cocktails. The adipogenic cocktails were modified by the addition of compounds to be investigated and rosiglitazone in the presence or absence of insulin. Results vividly showed that MHQ-1, MHQ-2 and MHQ-4 induced adipogenesis like insulin and enhanced adipogenesis like rosiglitazone significantly. Furthermore, MHQ1, MHQ2 and MHQ-4 as well as rosiglitazone as positive control were subjected to fluorescence glucose uptake test by 2-NBDG (fluorescent glucose analogue) on mature adipocytes. Results suggested significant glucose uptake activity by MHQ1, MHQ2 and MHQ-4. It is concluded and suggested that the further in-depth research study on the isolated compounds might help to discover a new safe compound with strong antidiabetic activity. Results further confirm the traditional use of *T. indica* in the management of diabetes in Malaysia.

ABSTRACT

التيتراسيرا انديكا مير(العائلة: الديلينية) أحد الأنواع الخشبية الكبيرة المتسلقة في الغابات المطيرة. تتواجد في كافة الغابات الماليزية، وتعرف محلياً باسم "ميميلاس بابا" أو نبات ورقة الرمل. الأجزاء الهوائية للتي. إنديكا استعملت قديماً في علاج العديد من الأمراض ومن ضمنها السكري والمشاكل المصاحبة له في ماليزيا. الإدعاءات القديمة على فعالية هذا النبات كعلاج للسكري لم تثبت علمياً بعد، لذلك هدفت هذه الدراسة لإستكشاف الخصائص المضادة للسكري لساق التي. إنديكا في الخلايا مخبرياً، بالإضافة لعزل المركبات المسؤولة عن الفعالية ضد السكري. دراسة الفعالية ضد السكري للمستخلص الايثانولي للساق والمستخلصات الفرعية والمركبات المعزولة من المستخلصات الفعالة أجريت على الخلايا الدهنية وقبل الدهنية نوع 3T3-L1. بعد البحث؛ أربعة فلافونويدات (-MHQ1-Wogonin, MHQ-2-Norwogonin, MHQ-3-Quercetin, MHQ-4-Teachtchrysin) واثنين من التيربينويدات (-MHQ-5-Stigmasterol, MHQ-6-Betulinic acid) تم عزلها من المستخلص الايثانولي للساق للتي. إنديكا، وتم فحص سميتها الخلوية عبر اختبارها في خلايا 3T3-L1 الدهنية فيما يتعلق بفعاليتها ضد السكري. جميع المركبات عزلت ونُقيت من خلال جل السيلكا والفصل باستعمال عمود sephadex LH-20 لعدة مرات، وتمت إعادة بلورتها بعدة مذيبات. تم الكشف عن تركيبها بواسطة أطياف 1H-and 13C-NMR. فحص سميتها على الخلايا تم بواسطة فحص MTT على خلايا 3T3-L1 قبل الدهنية للتحقق من الجرعة الآمنة للمستخلص والمستخلصات الفرعية والمركبات النقية مثل: MHQ1, MHQ2, MHQ3, MHQ4 والتي بدورها عُرِضت لعمليات بناء الدهون لدراسة النشاط المشابه للإنسولين أو النشاط المحفز لحساسية الإنسولين بغاية تقييم الفعالية المحتملة ضد السكري. جميع المركبات أدخلت للخلايا بعدة جرعات آمنة وأخرى محفزة لإنتاج الدهون. الجرعات المحفزة لإنتاج الدهون عُدلت بإضافة المركبات المراد دراستها إلى روزيغليتازون بوجود أو غياب الإنسولين. أظهرت النتائج بشكل واضح أن MHQ-1, MHQ-2, MHQ-4 and MHQ-4 تحفز إنتاج الدهون بشكل مماثل للإنسولين وكذلك روزيغليتازون. في سياق آخر، MHQ-1, MHQ-2 and MHQ-4 بالإضافة للروزيغليتازون كمجموعة تحكم عُرِضت لفحص أخذ الجلوكوز بالإشعاع بواسطة 2-NBDG على خلايا دهنية ناضجة. أظهرت النتائج إرتفاعاً ملحوظاً لأخذ الجلوكوز بواسطة MHQ1, MHQ2 وMHQ-4. نخلص مما سبق إلى ضرورة إستكمال البحث والدراسة على المركبات المعزولة والذي بدوره قد يساعد على اكتشاف مركبات جديدة وآمنة مع فعالية قوية ضد السكري. وكما أكدت الدراسة على الاستعمال التقليدي للتي. إنديكا في السيطرة على مرض السكري في ماليزيا.

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 of Pharmaceutical Chemistry.

.....
Qamar Uddin Ahmed
Supervisor

.....
Siti Zaiton Mat So'ad
Co-Supervisor

.....
Muhammad Taher
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 of Pharmaceutical Chemistry.

.....
Internal Examiner

.....
External Examiner

This thesis was submitted to the Department of Pharmacy and is accepted as a fulfilment of the requirement for the degree of Master of Pharmaceutical Chemistry.

.....
Siti Zaiton Mat So'ad.....
Head, Department of
Pharmaceutical Chemistry

This thesis was submitted to the Kulliyah of Pharmacy and is accepted as a fulfilment of the requirement for the degree of Master of Pharmaceutical Chemistry

.....

Siti Hadijah Shamsudin
Dean, Kulliyah of Pharmacy

DECLARATION

I hereby declare that this dissertation 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.

Md. Mahmudul Hasan

Signature

Date

COPYRIGHT PAGE

INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA

**DECLARATION OF COPYRIGHT AND AFFIRMATION OF
FAIR USE OF UNPUBLISHED RESEARCH**

**THE IMPACT OF MOBILE INTERFACE DESIGN ON
INFORMATION QUALITY OF M-GOVERNMENT SITES**

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

Copyright © 2016 (Md. Mahmudul Hasan) and International Islamic University Malaysia. All rights reserved.

No part of this unpublished research may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise without prior written permission of the copyright holder except as provided below

1. Any material contained in or derived from this unpublished research may be used by others in their writing with due acknowledgement.
2. IIUM or its library will have the right to make and transmit copies (print or electronic) for institutional and academic purposes.
3. The IIUM library will have the right to make, store in a retrieved system and supply copies of this unpublished research if requested by other universities and research libraries.

By signing this form, I acknowledged that I have read and understand the IIUM Intellectual Property Right and Commercialization policy.

Affirmed by Md. Mahmudul Hasan

.....
Signature

.....
Date

This thesis is dedicated to my parents for making me be who I am and my siblings for supporting me all the way!

ACKNOWLEDGEMENT

First of all, I praise Allah, the almighty, merciful and passionate, for providing me this opportunity and granting me the capability to finish my research successfully. This research work and thesis would not be able to complete without the continuous help, support and supervision of several people. I would like to express my gratitude to all of them.

It is my utmost pleasure to dedicate this work to my dear parents and my family, who granted me the gift of their unwavering belief in my ability to accomplish this goal: thank you for your support and patience.

I would like to express my sincere gratitude to Associate Professor Doctor Qamar Uddin Ahmed for his direct supervision and continuous efforts, helps and supports to finish the research and thesis. I would be quite impossible to finish the thesis on time without the kind support.

Additionally, a very special thanks to Associate Professor Doctor Siti Zaiton Mat So'ad and Associate Professor Doctor Muhammad Taher for their continuous support, encouragement and leadership, and for that, I will be forever grateful.

I wish to express my appreciation and thanks to those who provided their time, effort and support for this project specially Br. Tengku Muhamad Faris Syafiq and Br. Abdurrahman Abdul Kader. To the members of my dissertation committee, thank you for sticking with me.

TABLE OF CONTENTS

Abstract	ii
Abstract in Arabic	iii
Approval Page.....	iv
Declaration	vi
Copyright Page.....	vii
Dedication	viii
Acknowledgement	ix
Table of Contents	x
List of tables.....	xiv
List of Figures	xv
List of Symbols	xvii
List of Abbreviations	xviii
CHAPTER ONE: INTRODUCTION	1
1.1 Introduction.....	1
1.2 Problem Statement.....	3
1.3 Significance of the Study.....	4
1.4 Research Objectives.....	5
1.5 Theoretical Framework.....	5
1.6 Research Hypothesis.....	6
1.7 Research Questions.....	7
CHAPTER TWO: LITERATURE REVIEW.....	8
2.1 Diabetes Mellitus	8
2.2 Therapeutic Approach Towards Diabetes	9
2.3 Role of Plants in the Management of Disease	9
2.4 Medicinal Plants with Antidiabetic Effects:	11
2.5 Current Therapeutic Approach Towards Diabetes Mellitus	14
2.6 Flavonoids	16
2.6.1 Role of Flavonoids as Antidiabetic Agents.....	18
2.7 Assay of Insulin and Insulin-like Activity.....	21
2.7.1 Epididymal Fat Pad of Animals	22
2.7.2 Glucose Uptake in Adipocytes.....	23
2.8 Therapeutic Approach Towards Diabetes	23
2.9 Dilleniaceae	25
2.9.1 <i>Tetracera alnifolia</i> Willd.	27
2.9.2 <i>Tetracera volubilis</i> Linn.....	27
2.9.3 <i>Tetracera asperula</i> Miq.	27
2.9.4 <i>Tetracera costata</i> Mart, ex Eichler.	27
2.9.5 <i>Tetracera tigarea</i> Dc.....	26
2.9.6 <i>Tetracera macrophylla</i> Vall. (<i>T. monocarpa</i> , <i>T. Sarmentosa</i> Blanco.)	28
2.9.7 <i>Tetracera potatoria</i> Afzel. ex G. Don.....	28
2.9.8 <i>Tetracera scandens</i> Linn. Merr.....	28

2.9.9 <i>Tetracera indica</i> Merr	30
2.9.9.1 Classification of <i>T. indica</i>	31
2.9.9.2 Traditional uses of <i>T. indica</i>	31
2.9.9.3 Phytochemical and pharmacological evaluations of <i>T. indica</i>	32
CHAPTER THREE: METHODOLOGY.....	35
3.1 Chemicals	35
3.2 Instruments	35
3.3 Supplies Used	36
3.4 Collection of Plant Material.....	37
3.5 Preparation of Plant Material.....	38
3.6 Extraction of Plant Material.....	38
3.7 Fractionation of Ethanol Extract.....	39
3.8 Isolation of Compounds from the Ethyl Acetate Fraction.....	39
3.8.1 Fraction of Stems Ethanol Extract of <i>T. indica</i>	39
3.8.2 Isolation of Compounds of the Stems Ethanol Extract of <i>T. indica</i>	40
3.8.3 Cell Culture	41
3.8.4 MTT Viability Assay	41
3.8.4.1 Materials	41
3.8.4.2 Method.....	41
3.8.5 Differentiation / Adipogenesis	42
3.8.5.1 Materials	42
3.8.5.2 Methods	43
3.8.6 Fluorescence Glucose Uptake (GUT)	45
3.8.6.1 Materials	45
3.8.6.2 Method.....	45
3.8.7 Flow Chart of the <i>In-vitro</i> Studies	47
CHAPTER FOUR: RESULTS AND DISCUSSION.....	48
4.1 Structure Elucidation	48
4.1.1 Physical Properties of Isolated Compounds.....	48
4.1.2 Phytochemical Analysis.....	49
4.1.3 Isolation & Structure Elucidation of Purified Compounds of the Stems of <i>T. indica</i>	49
4.1.4 Isolation and Structure Elucidation of Purified Compounds from the Bioactive Fraction of the Stem of <i>T. indica</i>	50
4.1.5 Discussion	56
4.1.5.1 Spectroscopy.....	56
4.1.5.2 Isolation of compounds	56
4.2 <i>In-vitro</i> bioactivity determination.....	77
4.2.1 MTT Assay:	77
4.2.2 Differentiation/ Adipogenesis	79
4.2.2.1 Flow chart of adipogenesis:.....	79
4.2.3 Fluorescence glucose uptake.....	97
CHAPTER FIVE: CONCLUSIONS.....	101
REFERENCES:	103

APPENDIX A 1: 1H-NMR SPECTRUM OF WOGONIN (MHQ-1)	114
APPENDIX A 2: 1H-NMR SPECTRUM OF WOGONIN (MHQ-1)	115
APPENDIX A 3: 1H-NMR SPECTRUM OF WOGONIN (MHQ-1)	116
APPENDIX A 4: 13C-NMR SPECTRUM OF WOGONIN (MHQ-1).....	117
APPENDIX A 5: APT- 13C NMR SPECTRUM OF WOGONIN (MHQ-1).....	118
APPENDIX A 6: 13C-NMR SPECTRUM OF WOGONIN (MHQ-1).....	119
APPENDIX A 7: APT- 13C NMR SPECTRUM OF WOGONIN (MHQ-1).....	120
APPENDIX A 8: FTIR SPECTRUM OF WOGONIN (MHQ-1).....	121
APPENDIX A 9: MASS SPECTRUM OF WOGONIN (MHQ-1).....	122
APPENDIX B 1: 1H-NMR SPECTRUM OF NORWOGONIN (MHQ-2)	123
APPENDIX B 2: 1H-NMR SPECTRUM OF NORWOGONIN (MHQ-2)	124
APPENDIX B 3: 13C-NMR SPECTRUM OF NORWOGONIN (MHQ-2)	125
APPENDIX B 4: APT-13C NMR SPECTRUM OF NORWOGONIN (MHQ-2)....	126
APPENDIX B 5: APT-13C NMR SPECTRUM OF NORWOGONIN (MHQ-2)....	127
APPENDIX B 6: FTIR SPECTRUM OF NORWOGONIN (MHQ-2).....	128
APPENDIX B 7: MASS SPECTRUM OF NORWOGONIN (MHQ-2).....	129
APPENDIX C 1: 1H-NMR SPECTRUM OF QUERCETIN (MHQ-3)	130
APPENDIX C 2: 1H-NMR SPECTRUM OF QUERCETIN (MHQ-3)	131
APPENDIX C 3: 1H-NMR SPECTRUM OF QUERCETIN (MHQ-3)	132
APPENDIX C 4: 1H-NMR SPECTRUM OF QUERCETIN(MHQ-3)	133
APPENDIX C 5: APT- 13C-NMR SPECTRUM OF QUERCETIN(MHQ-3).....	134
APPENDIX C 6: APT- 13C-NMR SPECTRUM OF QUERCETIN (MHQ-3).....	135
APPENDIX C7: APT- 13C-NMR SPECTRUM OF QUERCETIN (MHQ-3).....	136
APPENDIX C 8: FTIR SPECTRUM OF QUERCETIN (MHQ-3)	137
APPENDIX C 9: MASS SPECTRUM OF QUERCETIN (MHQ-3).....	138
APPENDIX D 1: 1H NMR SPECTRUM OF TECHTOCHRYSIN (MHQ-4).....	139
APPENDIX D 2: 1H NMR SPECTRUM OF TECHTOCHRYSIN (MHQ-4).....	140
APPENDIX D 3: 1H NMR SPECTRUM OF TECHTOCHRYSIN (MHQ-4).....	141
APPENDIX D 4: 1H NMR SPECTRUM OF TECHTOCHRYSIN (MHQ-4).....	142
APPENDIX D 5: FTIR SPECTRUM OF TECHTOCHRYSIN (MHQ-4)	143
APPENDIX D 6: MASS SPECTRUM OF TECHTOCHRYSIN (MHQ-4).....	144
APPENDIX E 1: 1H NMR SPECTRUM OF STIGMASTEROL (MHQ-5)	145
APPENDIX E 2: FTIR SPECTRUM OF STIGMASTEROL (MHQ-5).....	146
APPENDIX F 1: IR SPECTRUM OF BETULINIC ACID (MHQ-6)	147
APPENDIX F 2: 1H NMR SPECTRUM OF BETULINIC ACID (MHQ-6).....	148
APPENDIX F 3: 1H NMR SPECTRUM OF BETULINIC ACID (MHQ-6).....	149
APPENDIX F 4: 1H NMR SPECTRUM OF BETULINIC ACID (MHQ-6).....	150
APPENDIX F 5: 1H NMR SPECTRUM OF BETULINIC ACID (MHQ-6).....	151
APPENDIX F 6: 1H NMR SPECTRUM OF BETULINIC ACID (MHQ-6).....	152
APPENDIX G 1: UV-SPECTRUM OF WOGONIN (MHQ-1).....	153
APPENDIX G 2: UV-SPECTRUM OF WOGONIN (MHQ-1).....	153
APPENDIX G 3: UV-SPECTRUM OF WOGONIN (MHQ-1).....	154
APPENDIX G 4: UV-SPECTRUM OF WOGONIN (MHQ-1).....	154
APPENDIX G 5: UV-SPECTRUM OF WOGONIN (MHQ-1).....	155
APPENDIX G 6 : UV-SPECTRUM OF WOGONIN (MHQ-1).....	155
APPENDIX G 7: UV-SPECTRUM OF WOGONIN (MHQ-1).....	156
APPENDIX G 8 : UV-SPECTRUM OF WOGONIN (MHQ-1).....	156
APPENDIX H 1: UV-SPECTRUM OF NORWOGONIN (MHQ-2)	157

APPENDIX H 2: UV-SPECTRUM OF NORWOGONIN (MHQ-2)	157
APPENDIX H 3: UV-SPECTRUM OF NORWOGONIN (MHQ-2)	158
APPENDIX H 4: UV-SPECTRUM OF NORWOGONIN (MHQ-2)	158
APPENDIX H 5: UV-SPECTRUM OF NORWOGONIN (MHQ-2)	159
APPENDIX H 6: UV-SPECTRUM OF NORWOGONIN (MHQ-2)	159
APPENDIX H 7: UV-SPECTRUM OF NORWOGONIN (MHQ-2)	160
APPENDIX H 8: UV-SPECTRUM OF NORWOGONIN (MHQ-2)	160
APPENDIX I 1: UV SPECTRUM OF NORWOGONIN (MHQ-2)	161
APPENDIX I 2: UV SPECTRUM OF QUERCETIN (MHQ-3)	161
APPENDIX I 3: UV SPECTRUM OF QUERCETIN (MHQ-3)	162
APPENDIX I 4: UV SPECTRUM OF QUERCETIN (MHQ-3)	162
APPENDIX I 5: UV SPECTRUM OF QUERCETIN (MHQ-3)	163
APPENDIX I 6: UV SPECTRUM OF QUERCETIN (MHQ-3)	163
APPENDIX I 7: UV SPECTRUM OF QUERCETIN (MHQ-3)	164
APPENDIX I 8: UV SPECTRUM OF QUERCETIN (MHQ-3)	164
APPENDIX J 1: UV-SPECTRUM OF TECHTOCHRYSIN (MHQ-4)	165
APPENDIX J 2: UV-SPECTRUM OF TECHTOCHRYSIN (MHQ-4)	165
APPENDIX J 3: UV-SPECTRUM OF TECHTOCHRYSIN (MHQ-4)	166
APPENDIX J 4: UV-SPECTRUM OF TECHTOCHRYSIN (MHQ-4)	166
APPENDIX J 6: UV-SPECTRUM OF TECHTOCHRYSIN (MHQ-4)	167
APPENDIX J 7: UV-SPECTRUM OF TECHTOCHRYSIN (MHQ-4)	167
APPENDIX J 8: UV-SPECTRUM OF TECHTOCHRYSIN (MHQ-4)	168
APPENDIX K 1: MTT VIABILITY ASSAY OF ETHANOL EXTRACT.....	169
APPENDIX K 2: MTT VIABILITY ASSAY OF ETHANOL EXTRACT.....	170
APPENDIX K 3: MTT VIABILITY ASSAY OF HEXANE FRACTION	171
APPENDIX K 4: MTT VIABILITY ASSAY OF HEXANE FRACTION	172
APPENDIX K 5: MTT VIABILITY ASSAY OF ETHYL ACETATE FRACTION	173
APPENDIX K 6: MTT VIABILITY ASSAY OF ETHYL ACETATE FRACTION	174
APPENDIX K 7: MTT VIABILITY ASSAY OF WOGONIN (MHQ-1)	175
APPENDIX K 8: MTT VIABILITY ASSAY OF WOGONIN (MHQ-1)	176
APPENDIX K 9: MTT VIABILITY ASSAY OF NORWOGONIN (MHQ-2).....	177
APPENDIX K 10: MTT VIABILITY ASSAY OF NORWOGONIN (MHQ-2).....	178
APPENDIX K 11: MTT VIABILITY ASSAY OF QUERCETIN (MHQ-3).....	179
APPENDIX K 12: MTT VIABILITY ASSAY OF QUERCETIN (MHQ-3).....	180
APPENDIX K 13: MTT VIABILITY ASSAY OF TECHTOCHRYSIN (MHQ-4)	181
APPENDIX K 14: MTT VIABILITY ASSAY OF TECHTOCHRYSIN (MHQ-4)	182
APPENDIX K 15: MTT VIABILITY ASSAY OF STIGMASTEROL (MHQ-5) ...	183
APPENDIX K 16: MTT VIABILITY ASSAY OF STIGMASTEROL (MHQ-5) ...	184
APPENDIX K 17: MTT VIABILITY ASSAY OF BETULINIC ACID (MHQ-6)..	185
APPENDIX K 18: MTT VIABILITY ASSAY OF BETULINIC ACID (MHQ-6)..	186

LIST OF TABLES

<u>Table No.</u>	<u>Page No.</u>
Table 2.1: Medicinal plants with antidiabetic effects.	12
Table 2.2: List of drugs used in the treatment of Diabetes Mellitus with their mechanism of action (MOA) and risk factors.....	15
Table 2.3: Nomenclature of the subclasses of flavonoids based on the position of their substituents.....	17
Table 2.4: Some therapeutic approaches in animal model to treat diabetes mellitus by the isolated flavonoids	20
Table 2.5: Assay of insulin and insulin-like activity	21
Table 3.1: Adipogenic cocktail; three safe concentration of the compounds were applied in the microplate which was determined according to the safety of the cells in MTT assay.	43
Table 3.2: 2-NBDG uptake groups; three concentrations of were used according to the safety of the cells determined in MTT assay.	46
Table 4.1: Physical properties of isolated biactive compounds	48
Table 4.2: ^1H (600 MHz) and ^{13}C -NMR (150 MHz) chemical shifts of compound MHQ-1 [δ (ppm) Acetone- d_6 , coupling constants (J) in Hz] in comparison with (Harrison et al., 1994).	59
Table 4.3: ^1H (600 MHz) and ^{13}C -NMR (150 MHz) chemical shifts of compound MHQ-2 [δ (ppm) Acetone- d_6 , coupling constants (J) in Hz]	63
Table 4.4: ^1H (600 MHz) and ^{13}C -NMR (150 MHz) chemical shifts of compound MHQ-3 [δ (ppm) Acetone- d_6 , coupling constants (J) in Hz] (Chang et al., 2009).....	67
Table 4.5: ^1H (600 MHz) and ^{13}C -NMR (150 MHz) chemical shifts of compound MHQ-4 [δ (ppm) MeOD- d_4 , coupling constants (J) in Hz] (Sutthanut et al., 2007)....	70
Table 4.6: ^1H (600 MHz) and ^{13}C -NMR (150 MHz) chemical shifts of compound MHQ-5 [δ (ppm) CDCl_3 - d_3 , coupling constants (J) in Hz].	73
Table 4.7: ^1H (600 MHz) and ^{13}C -NMR (150 MHz) chemical shifts of compound MHQ-6 [δ (ppm) CDCl_3 - d_3 , coupling constants (J) in Hz] in comparison with (Ahmed et al., 2014).	76

List of Figures

Figure No:	Page No.
Figure 2.1: Discovery of Metformin was done from aerial part of <i>Galega officinalis</i> .	10
Figure 2.2. Parent structure of flavonoids.....	16
Figure 2.3: Warburg vessel used to measure the glucose uptake of the media.....	23
Figure 2.4: Isolated compounds from <i>T. indica</i>	32
Figure 2.5: <i>Tetracera indica</i> Merr	34
Figure 3.1: Isolation of compounds from ethanol extract of the stem of <i>T. indica</i>	40
Figure 3.2: Flow chart of adipogenesis.....	44
Figure 3.3: 2-NBDG uptake treatment groups.....	46
Figure 3.4: Flow chart of the whole study.	47
Figure 4.1: Chemical structure of wogonin (5, 7-dihydroxy-8-methoxyflavone) (MHQ-1)	59
Figure 4.2: Norwogonin; 5,7,8-trihydroxyflavone (MHQ-2)	62
Figure 4.3: MHQ-3 as Quercetin (2-(3, 4-Dihydroxyphenyl)-3, 5, 7 trihydroxy-4H-1-benzopyran-4-one; 3', 4', 3, 5, 7-pentahydroxyflavone; C ₁₅ H ₁₀ O ₇).....	66
Figure 4.4: Techtochrysin; 5-hydroxy-7-methoxyflavone) (MHQ-4).....	69
Figure 4.5: MHQ-5 as (24R)-24-ethyl-5a-cholesta 5, 22-dien-3β-ol (Stigmasterol)...	72
Figure 4.6: Betulinic acid, (3β)-3-Hydroxy-lup-(20) (29) en-28-oic acid, (MHQ-6)..	75
Figure 4.8: Step by step progression of adipogenesis.....	79
Figure 4.9: Oil-red-O staining of 3T3-L1 adipocytes on day 10.	80
Figure 4.10: <i>T. indica</i> stems ethanol extract, it's two fractions (Hexane and ethyl acetate) and isolated four compounds (MHQ-1, MHQ-2, MHQ-3, MHQ-4).....	81
Figure 4.11: <i>T. indica</i> stem 95% ethanol extract.	84
Figure 4.12: <i>T. indica</i> stem ethanol extract.....	85

Figure 4.13: Hexane fraction of <i>T.indica</i> stem ethanol extract.....	86
Figure 4.14: Ethyl acetate fraction of <i>T.indica</i> stem ethanol extract.	87
Figure 4.15: Ethyl acetate fraction of <i>T.indica</i> stem ethanol extract.	88
Figure 4.16: MHQ-1 (Wogonin) isolated from the ethyl acetate fraction of <i>T. indica</i> stem ethanol extract.	89
Figure 4.17: MHQ-1 (Wogonin) isolated from the ethyl acetate fraction of <i>T. indica</i> stem ethanol extract	90
Figure 4.18: MHQ-2 (Norwogonin) isolated from the ethyl acetate fraction of <i>T. indica</i> stem ethanol extract.	91
Figure 4.19: MHQ-2 (Norwogonin) isolated from the ethyl acetate fraction of <i>T. indica</i> stem.	92
Figure 4.20: MHQ-3 isolated from the ethyl acetate fraction of <i>T. indica</i> stem ethanol extract.....	93
Figure 4.21: MHQ-3 isolated from the ethyl acetate fraction of <i>T. indica</i> stem ethanol extract.....	94
Figure 4.22: MHQ-4 (Techtochrysin) isolated from the ethyl acetate fraction of <i>T. indica</i> stem ethanol extract.	95
Figure 4.23: MHQ-4 (Techtochrysin) isolated from the ethyl acetate fraction of <i>T. indica</i> stem ethanol extract	96
Figure 4.24: MHQ-1 (Wogonin), MHQ-2 (Norwogonin) and MHQ-4 (Techtochrysin) were evaluated to stimulate 2-NBDG uptake in 3T3-L1 adipocytes	99

LIST OF SYMBOLS

d	Doublet
Hz	Hertz
J	Coupling Constant
Kg	Kilogram
m	Multiplet
mg	Milligram
s	Singlet
μg	Microgram
%	Percentage
*P	P value <0.05
**p	P value <0.005

LIST OF ABBREVIATIONS

ACETONE-D ₆	Deuterated Acetone
APT	Attached Proton Test
CDCl ₃	Deuterated chloroform
DCM	Dicholoro Methane
DM	Diabetes Mellitus
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl Sulfoxide
DPP	Dipeptidyl Peptidase
EtOH	Ethanol
FTIR	Fourier transform infrared spectroscopy
GLUT	Glucose Transporter
IC ₅₀	Inhibitory Concentration where the response reduced by half
MOA	Mechanism of Action
MTT	3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide
NHMS	National Health and Morbidity Survey
ORO	Oil Red O Staining
PBS	Phosphate Buffered Saline
PPM	Parts Per Million
SPSS	Statistical Package for the Social Sciences
TEF	Toluene: Benzene: Formic Acid
TLC	Thin Layer Chromatography
¹ H NMR	Proton Nuclear Magnetic Resonance
¹³ C NMR	¹³ Carbon Nuclear Magnetic Resonance
2-NBDG	2-(N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl) Amino)-2-Deoxyglucose

CHAPTER ONE

INTRODUCTION

1.1 INTRODUCTION

Diabetes mellitus is a metabolic disorder which gradually leads a person to death. In Malaysia, the rate of DM is dramatically increasing and therefore it has become a major threat to all Malaysians of different age groups. The prevalence of diabetes in Malaysia was recorded 0.65% in 1960. However, according to the First National Health and Morbidity Survey, the DM prevalence was found to be 6.3% in 1986 which was further increased to 8.2% in 1996 (NHMS II). More interestingly, the recent survey revealed very appalling information as the prevalence of DM was found to be about 20.8% which is astonishingly an increase of 80% in a decade at 8% rising rate every year (NHMS I & NHMS II). This indicates that, the prevalence of DM in Malaysia is alarming and has doubled in the last decade (NHMS III). Another major concern about DM is diabetes comorbidity (Meyers et al., 2014). DM leads to many other complications like cardiovascular diseases, obesity, hypoglycemic shock etc. which collectively responsible to decrease the quality of life and eventually lead to death (Abdelmalik et al., 2007).

In Malaysia, the similar scenario has been observed as there is a gradual increase of diabetes rate among the Malaysian adults aged 30 years or above from 6.3 to 8.3 in the year 1986 and 1996 which reached to 14.9% in 2006 (Nazaimoon et al., 2013). Since DM is a chronic disorder, it creates many health complications which lead to financial crisis to the patients. Apart from using insulin, many oral medications are utilized in the management of DM. Most of the medications used in DM treatment have been reported

to exert deleterious side effects and they are costly too. At some point of the prolonged period of DM treatment, people either stop taking prescribed synthetic medicines or diverted to herbal medicines (Ettaro et al., 2004).

The search for a new class of safe antidiabetic agents is regarded as an important scientific endeavor to overcome chronic diabetes and its related infirmities. Therefore, there is always a continuous search for alternative drugs. Medicinal plants are considered the best source to obtain a variety of drugs according to the World Health Organization (Farnsworth et al., 1985). With respect to treat hypoglycaemic as well as hyperglycaemic conditions, many medicinal plants are used and referred to considerable interest to ethno-botanical community as they are recognized to contain valuable and important medicinal properties in different parts of the plant and also a number of plants have shown varying degrees of hypoglycaemic and anti-hyperglycaemic effects as well (Ponnusamy et al., 2011). Many medicinal plants have been reported to afford active principles with desired pharmacological properties to cure ailments such as type-1 and type-2 DM (Fabricant & Farnsworth, 2001).

At present, researchers have become more interested in plant source and a numerous biological studies are currently undergoing in regard to find out safe and effective antidiabetic agents from plants source. In this regard, *Tetracera indica* (Houtt. Ex Christm. & Panz.) Merr. (Dilleniaceae) is one of the Malaysian plants to address this issue effectively. It is a large, woody, rain forest climber of Malaysia which is commonly known as mempelas paya or sand paper plant. It has white colored flower and leaves are simple and medium shaped. It has berry-like fruits which are sour in taste (Christophe, 2002). Different parts of the *T. indica* have been used for healing of fever, flue, sinus symptoms, skin rashes, itching, piles, mouth ulcer, diarrhea, insects bites and diabetes. *T. indica* is also used as one of the active ingredients in a local herbal drug viz,

Plantisol, which is widely prescribed and recommended to effectively manage diabetes in Malaysia by the local herbalist practitioners. *Barringtonia racemosa*, *Pithecellobium jiringa*, *Tinospora crispa* and *Andrographis paniculata* are other active ingredients of Plantisol (<http://www.klik4sihat.com/kencingmanis>). In this research, our aim is to evaluate an *in-vitro* antidiabetic potential of the stems of *T. indica* and isolated compounds from the stems extract with respect to find out safe and efficacious antidiabetic agents.

1.2 PROBLEM STATEMENT

Currently available therapeutic options for non-insulin-dependent diabetes mellitus (NIDDM) such as dietary modification, oral hypoglycemic, and insulin are not only costly to manage but have limitations of their own. Many of these antidiabetic agents have a number of serious toxic side effects on health; thus management of diabetes without any side effects is still considered as a great challenge to tackle. Hence, the search for more effective and safer antidiabetic agents (i.e. antihyperglycemic agents) has continued to be an important area of investigation for scientists throughout the world. There has been a growing interest in antidiabetic agents from natural products, particularly those derived from traditional medicinal plants. Currently available therapies for diabetes include insulin and various oral antidiabetic agents such as sulfonylureas, biguanides and α -glucosidase inhibitors which are used as monotherapy or in combination to achieve better glycemic regulation. Many of these oral anti-diabetic agents suffer from various deleterious effects, thus, managing diabetes without any side effects is still a great challenge to the scientists (Ahmed et al., 2012), and hence the search for more effective and safer therapeutic agents in managing diabetes has continued to be an important area of investigation. In this regard, *T. indica* could be one

of the candidates to tackle aforementioned problems associated with DM. Aerial parts of *T. indica* are used in the management of diabetes in different parts of Malaysia. However, antidiabetic potential of the compounds present in the aerial parts of *T. indica* is yet to be scientifically evaluated appropriately. Therefore, this research work will highlight the development of the compounds present in the stems of *T. indica* into a widely used safe antidiabetic drug to control or prevent diabetes efficaciously. This study will provide preclinical evidence and elucidate the extent of pharmacological activities of the compounds present in the stems of *T. indica* which could prove to be a potential clinical drug in the management of diabetes.

1.3 SIGNIFICANCE OF THE STUDY

In United States, 56.9% people with diagnosed diabetes take only oral medications (Diabetes statistics report, 2010-12; CDC). As diabetes is considered a chronic disease with comorbidity, it is a major financial threat for the patients and their families as well. The oral medications available in the market are also not free of side effects and they are costly too. So patients are becoming more interested in the traditional medicines which are mainly prepared from the plants extracts of traditional medicinal plants widely used to cure various diseases throughout the world (Christensen et al., 2009).

Different parts of *T. indica* have been traditionally used to manage DM in Malaysia. However, no attempt has been made to discover biologically active compounds from this plant. Hence, our aim in this research study is to explore *in vitro* antidiabetic potential of the stems of *T. indica* and isolate active principles responsible for the *in-vitro* antidiabetic activity of the stems of *T. indica*. Through meticulous investigation on the isolated compounds and *in-vitro* antidiabetic activity testing, we anticipate to find out the phytoconstituents which could be responsible for the

antidiabetic activity of the stems of *T. indica* and the resultant compounds showing promising *in vitro* antidiabetic effect might provide lead for the discovery of safe antidiabetic agents in the management of diabetes.

1.4 RESEARCH OBJECTIVES

The objective of the research was as follows:

1. To investigate an *in vitro* antidiabetic activity of the stems ethanol extract of *T. indica* on 3T3-L1 adipocytes.
2. To isolate phytoconstituents of the stems ethanol extract of *T. indica*.
3. To check the cytotoxicity of the fractions and isolated compounds on 3T3-L1 pre-adipocytes.
4. To investigate *in-vitro* antidiabetic activity of all isolated compounds on 3T3-L1 adipocytes.

1.5 THEORETICAL FRAMEWORK

For type II diabetes mellitus several types of therapeutic approaches are taken into account viz. increase insulin secretion by pancreas, increase insulin sensitivity to target organ or increase glucose uptake to adipocyte cells. In this study, insulin-like and insulin-sensitizing activity of the isolated compounds has been checked in both pre-adipocytes and adipocytes. MTT viability assay has been followed to determine the concentration to which the compound is toxic to the cells. Later on, activity of the compounds at safe concentration has been checked on 3T3-L1 adipocytes. Adipogenesis is a complex process where pre-adipocytes become mature with hundreds

of genes alteration that lead to reduction of glucose level in blood (Rosen & Spiegelman, 2000).

Again, glucose uptake of mature adipocytes is stimulated by insulin through re-localization of glucose transporter type 4 (GLUT 4) from intracellular stores to the plasma membrane (Kuppusamy et al., 2014). Glucose uptake was measured by a newly developed fluorometric method whereby a fluorescence analog of glucose 2-NBDG was used (Manaharan et al., 2013). All the mechanisms mentioned were investigated on the isolated compounds from *T. indica*. The structure of the isolated compound has been elucidated through extensive NMR studies which shed some light on further structural modification of the biologically active compounds.

The main objective of the study was to investigate the antidiabetic effect of the stems of *T. indica* and isolated compounds from the stems ethanol extract and finding out the mechanism of action of ethanol extract as well as all the isolated compounds from the same extract on 3T3-L1 adipocytes.

1.6 RESEARCH HYPOTHESIS

The primary hypothesis of the study was as follows:

H1: *T. indica* is traditionally used in the management of diabetes in Malaysia and contains antidiabetic agents.

The specific hypothesis of the study is given below:

H2: *T. indica* and isolated compounds induce adipogenesis like insulin.

H3: *T. indica* and isolated compounds enhance adipogenesis and have insulin sensitizing activity like rosiglitazone.

H4: *T. indica* and isolated compounds can uptake fluorescent glucose analog (2-NBDG) like insulin.