View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Repository@USM

EVALUATION OF ANTIHYPERGLYCEMIC EFFECTS, NUTRITIONAL/ANTINUTRITIONAL VALUES AND PHYTOCHEMICAL CONTENTS OF *OCIMUM TENUIFLORUM* LINN

LEILA MOUSAVI

UNIVERSITI SAINS MALAYSIA

2018

EVALUATION OF ANTIHYPERGLYCEMIC EFFECTS, NUTRITIONAL/ANTINUTRITIONAL VALUES AND PHYTOCHEMICAL CONTENTS OF *OCIMUM TENUIFLORUM* LINN

by

LEILA MOUSAVI

Thesis submitted in fulfilment of the requirements

for the degree of

Doctor of Philosophy

April 2018

ACKNOWLEDGEMENT

First and foremost, I offer my sincerest gratitude to my mother, father, my lovely family and my dearest and lovely little daughter, Selena who has supported me throughout this stage of my life unsparingly and I attribute my thesis to them.

Furthermore, I am deeply grateful and indebted to my supervisor Dr. Rabeta Mohd Salleh, my co-supervisor Associate Professor Dr. Vikneswaran Murugaiyah and special thanks to Professor Dr. Mohd. Zaini Asmawi for their help and support throughout this wonderful and often overwhelming experience**.**

I would like to extend my gratitude to dean of School of Industrial Technology for providing the financial support under Graduate assistance (GA) and great academic support during my study. Furthermore, I would like to forward my sincere thanks to Universiti Sains Malaysia for providing Postgraduate Fellowship and Malaysian Ministry of hight education for Awarded Malaysian International Scholarship (MIS) and also Universiti Sains Malaysia Short Term (304/PTEKIND/6312124) and RU grants (1001/PTEKIND/6711526) that contributed to the fundings of this study. In addition, my appreciations go to the staff at Food Technology Division, School of Pharmaceutical Science and School of Biology, USM who have made my stay enjoyable and memorable.

Many thanks to my friends and my laboratory mates especially Boshra Varastegani, Muhammad Ezzudin, Muhammad Qasimie, Rohini, Raghdaa, Maryam Sharafinejad, Bassel, Idris, Majeed, Abu Baker, Abdul and Elham.

Lastly, I offer my sincere thanks and blessings to Malaysia, the great land with peace and kind people who give me a lot of things under the shadow of the safe, peace and love.

TABLES OF CONTENTS

ACKNOWLEDGEMENT ii

TABLE OF CONTENTS iii

LIST OF TABLES xv

LIST OF FIGURES xx

LIST OF ABBREVIATIONS xxiv

ABSTRAK xxvii

ABSTRACT xxxi

CHAPTER 1 – INTRODUCTION

1.1 Background 1

1.2 Aims and objectives 6

1.3 Conceptual framework 9

CHAPTER 2 – LITERATURE REVIEW

2.1 Medicinal Plant 11

2.2 Botany of *Ocimum tenuiflorum* 15

2.2.1 Micronutrient status in diabetes mellitus 17

2.2.2 Phytochemicals and bioactive compounds constituents 18

2.3 Cytotoxic effects of medicinal plant 21

2.4 Diabetes 22

2.4.1 Anti-diabetic compounds from natural product 24

2.5 Plant material and extraction 26

2.5.1 Plant distribution 26

2.5.2 Choice of solvent 27

2.6 The role of phytochemical in glucose metabolism 33

2.6.1 Alpha-amylase and Alpha-glucosidase inhibition 36

2.6.2 Blood glucose response 37

2.6.3 Prevention of type 2 diabetes development 38

2.6.4 Major organs affected by diabetes mellitus 38

2.6.5 Conventional management of diabetes mellitus 42

2.6.6 Essential anti-diabetic mineral elements from herbs 45

2.6.7 Essential vitamins with hypoglycemic activity from herbs 48

2.6.7(a) Retinol 49

2.6.7(b) Alpha-tocopherol 50

2.6.7(c) Thiamine 52

2.6.7(d) Nicotinic acid (Niacin) and Nicotinamide 53

2.6.7(e) Ascorbic acid 54

CHAPTER 3 – GENERAL MRTHODOLOGY

3.1 Material and Methods 59

3.1.1 Plant material and preparation of sample 59

3.1.2 Extraction method 59

3.1.3 Preparation of fractions 60

3.2 Chemical, equipments and standard solutions 62

3.3 Preparations of chemicals, reagents, buffers and drug/extracts 62

3.3.1 Experimental animal and induction of experimental diabetic rat 63

3.3.2 Preparations of streptozocin model of diabetic mellitus 64

3.4 Blood sample collection 64

3.4.1 Intra-cardiac method for blood collects from the heart 65

3.4.2 Procedure for euthanizing the animal using carbon dioxide 65

3.4.3 Procedures for tail vein 65

3.5 General statistical procedure 66

CHAPTER 4 – DETERMINATION OF THE NUTRITIONAL AND ANTINUTRITIONAL VALUES OF THE LEAVES AND STEMS OF *OCIMUM TENUIFLORUM* LINN

4.1 Introduction 67

4.2 Objectives 70

4.3 Material and Methods 71

4.3.1 Plant material and preparation of sample 71

4.3.2 Chemical, equipments and standard solutions 71

4.3.2(a) Proximate analyze 71

4.3.2(b) Fatty acid analyze 71

4.3.2(c) Amino acids analysis 72

4.3.2(d) Water soluble and Fat soluble vitamin analyze 73

4.3.3 Phytochemical determination 74

4.3.3(a) Total saponin determination 74

4.3.3(b) Total phytic acid determination 75

4.3.3(c) Determination of flavonoid 76

4.3.3(d) Determination of alkaloids 76

4.3.3(e) Determination of total tannin by titration 77

4.3.3(f) Determination of total phenols by spectrophotometric method 77

4.4 Statistical analysis 78

4.5 Results and Discussion 78

4.5.1 Proximate composition of *Ocimum tenuiflorum* L. leaves and stems 78

4.5.2 Fatty acid profile of *Ocimum tenuiflorum* L. leaves and stems 82

4.5.3 Amino acid profile of *Ocimum tenuiflorum* L. leaves and stems 86

4.5.4 Vitamin compositions of *Ocimum tenuiflorum* L. leaves and stems 88

4.5.5 Saponin, Phytic acid of *Ocimum tenuiflorum* L. leaves and stems 90

4.5.6 Phytochemical contents of *Ocimum tenuiflorum* L. leaves and stems 93

4.6 Conclusion 96

CHAPTER 5 – ANTIHYPERGLYCEMIC EFFECT OF ORALLY ADMINSTERED ALCOHOLIC EXTRACTS OF *OCIMUM TENUIFLORUM* LINN IN NORMAL AND STREPTOZOTOCIN INDUCED DIABETIC RATS

5.1 Introduction 97

5.2 Objectives 99

5.3 Material and Methods 99

5.3.1 Plant material and extraction procedure 99

5.3.2 Experimental animal and induction of experimental diabetic rats 99

5.3.3 Blood sample collection 99

5.3.4 Chemical and equipments 99

5.4 Experimental Design 99

5.4.1 Subcutaneous glucose tolerance test (SGTT) on the normal and STZ-induced rats 100

5.4.2 The effect of single dose oral administration of the five extracts of *Ocimum tenuiflorum* on the normal and STZ-induced rats 100

5.4.3 The antihyperglycemic effect of daily oral administration of the five extracts of *Ocimum tenuiflorum* L. on 101

STZ-induced rats

5.4.4 Determination of the total phenolic content 101

5.4.5 Determination of the total flavonoid content 101

5.5 Statistical analysis 102

5.6 Results and Discussion 102

5.6.1 The effect of subcutaneous glucose tolerance test (SGTT) on the normal and STZ-induced rats 102

5.6.2 The effect of single dose oral administration of the five extracts of *Ocimum tenuiflorum* Linn. on the normal and STZ-induced rats 106

5.6.3 The antihyperglycemic effect of repeated daily oral administration of the five extracts of *Ocimum tenuiflorum* leaves on STZ-induced rats and body weight 109

5.6.4 Total phenolics in *Ocimum tenuiflorum* leaves extracts 112

5.6.5 Total flavonoids in *Ocimum tenuiflorum* leaves extracts 112

5.7 Conclusion 117

CHAPTER 6 – PHYTOCHEMIAL STUDY OF ACTIVE CRUDE EXTRACT OF *OCIMUM TENUIFLORUM* LINN AND IT'S FRACTIONS

6.1 Introduction 119

6.2 Objectives 122

6.3 Material and Methods 123

6.3.1 Preparation of the *Ocimum tenuiflorum* Lnn. extracts and fractions 123

6.3.1(a) Chemical and equipments 123

6.3.1(b) Preparation of the extracts (Serial Extraction) 123

6.3.1(c) Preparation of the fractions 123

6.3.2 Phytochemical screening of crude extracts of *Ocimum tenuiflorum* Lnn. leaves and methanol fractions 123

6.3.2(a) Identification by chemical test 123

6.3.2(a)(i) Identification of free reducing sugars 123

6.3.2(a)(ii) Identification of tannins 124

6.3.2(a)(iii) Identification of steroid (Liberman-buchard test) 124

6.3.2(a)(iv) Identification of terpenoids 124

6.3.2(a)(v) Identification of flavonoids (Ferric chloride test) 124

6.3.2(a)(vi) Identification of soluble starch 124

6.3.2(a)(vii) Identification of saponin 125

6.3.2(a)(viii) Identification of glycoside 125

6.3.2(b) Identification by Liquid Chromatography 125

Mass Spectrometry (LCMS)

6.3.2(b)(i) Chromatographic conditions 125

6.3.2(b)(ii) Mass Spectrometric Parameters 126

6.3.2(b)(iii) Sample preparation 126

6.4 Statistical analysis 126

6.5 Results and Discussion 126

6.5.1 Extraction, fraction yield 126

6.5.2 Phytochemical identification of crude extracts and fractions by chemical test 129

6.5.3 Identification by Liquid Chromatography Mass Spectrometry (LCMS) 131

6.6 Conclusion 139

CHAPTER 7 – ACUTE TOXICITY STUDY OF *OCIMUM TENUIFLORUM* LINN LEAVES CRUDE EXTRACTS

7.1 Introduction 140

7.2 Objectives 143

7.3 Material and Methods 144

7.3.1 Plant material and extraction procedure 144

7.3.2 Experimental animals 144

7.3.3 Histopathology assessment 145

7.3.3(a) Specimen collection and preparation 145

7.3.3(b) Specimen preparation 147

7.3.3(c) Hematoxyllin and eosin staining (H&E) 147

7.3.4 Acute toxicity study 149

7.3.4(a) Effects on vital organs: following the sacrifice 149

7.3.4(b) Biochemical and haematology parameters 150

7.4 Statistical Analysis 150

7.5 Results and Discussion 151

7.5.1 Effects of different extracts of *Ocimum tenuiflorum* L. on organ weights in STZ-induced treated rats 151

7.5.2 Body weight and behavioral observations of acute toxicity study 153

7.5.3 Histology assessment 155

7.5.3(a) Histopathology of kidneys 155

7.5.3(b) Histopathology of liver 158

7.5.3(c) Histopathology of the pancreas 161

7.5.4 Biochemical parameters 164

7.5.4(a) Effects of different extracts of *Ocimum tenuiflorum* L. leaves on the plasma lipid profiles of STZ-induced diabetic rats 164

7.5.4(b) Effects of oral administration of crud extracts of *Ocimum tenuiflorum* L. leaves on plasma hepato-specific markers and plasma electrolyte profiles of STZ-inducedrats 166

7.5.4(c) Effects of oral administration of crud extracts of *Ocimum tenuiflorum* L. leaves on liver function profiles of STZ-induced rats 169

7.5.5 Haematology parameters 171

7.5.5(a) Effects of the chloroform, ethyl acetate, methanol and water extracts of the leaves of *Ocimum tenuiflorum* L. on the haematological indices of STZ-induced rats 171

7.6 Conclusion 177

CHAPTER 8– ANTIDIABETIC AND *IN VITRO* ENZYME INHIBITION STUDIES OF ACTIVE CRUDE EXTRACT OF *OCIMUM TENUIFLORUM* LINN AND ITS FRACTIONS

8.1 Introduction 178

8.2 Objectives 181

8.3 Material and Methods 182

8.3.1 Preparation of the *Ocimum tenuiflorum* Lnn. extracts and fractions 182

8.3.2 Experimental animal and induction of diabetes 182

8.3.3 Blood sample collection and chemical and drugs used 182

8.3.4 Evaluation of antidiabetic activity of different dose of methanol extract of the *Ocimum tenuiflorum* Lnn. leaves 182

8.3.5 Evaluation of antidiabetic activity of single dose of different methanol fractions of the *Ocimum tenuiflorum* Lnn. leaves 183

8.3.6 *In vitro* α-glucosidase inhibition assay 183

8.3.6(a) Experimental set up 183

8.3.7 *In vitro* α-amylase inhibition assay 184

8.3.7(a) Preparation of solutions 184

8.3.7(b) Experimental set up 185

8.3.8 Insulin measurement 186

8.3.8(a) The principles of the assay 186

8.4 Results and Discussion 189

8.4.1 Effect of different doses of methanolic extract on blood glucose levels and body weight of STZ-induced diabetic rat 189

8.4.2 Effect of different doses of methanolic fractions on blood glucose levels and body weight of STZ-induced diabetic rat 192

8.4.3 *In vitro* α-glucosidase inhibition activity 195

8.4.4 *In vitro* α-amylase inhibition activity 198

8.4.5 Insulin level 201

8.5 Conclusion 208

CHAPTER 9 – CONCLUSION AND RECOMMENDATION

9.1 Conclusion 209

9.2 Further Recommendation 211

REFERENCES 212

APPENDICES

LIST OF PUBLICATIONS

LIST OF TABLES

		Page
Table 1.1	Top 10 countries for diabetes prevalence in 2010 and 2030	2
Table 2.1	Micronutrient status of diabetic patients	18
Table 2.2	Common solvents use for extraction	29
Table 2.3	Solvents used for active component extraction	32
Table 4.1	Proximate composition of *Ocimum tenuiflorum* L. leaves and stems (% d.b.)	81
Table 4.2	Fatty acid profile of of *Ocimum tenuiflorum* L. leaves and stems	84
Table 4.3	Amino acid profile of *Ocimum tenuiflorum* L. leaves and stems	87
Table 4.4	Vitamin compositions of *Ocimum tenuiflorum* L. leaves and stems	89
Table 4.5	Phytic acid, saponin of *Ocimum tenuiflorum* L. leaves and stems	92
Table 4.6	Phytochemical constituents on dry weight basis expressed as mg/100g	95
Table 6.1	Crude extracts (%) yield of *Ocimum tenuiflorum* L. leaves and stems (100g freeze dried sample)	127
Table 6.2	Fractions of methanol crude extract of *Ocimum tenuiflorum* L. leaves (100g)	128

Table 6.3	Phytochemical screening of HE, CF, EA, ME, AQ of *Ocimum tenuiflorum* L. leaves	129
Table 6.4	Phytochemichal screening of CF, BU, EA, ETH and AQ	130
Table 6.5	Phytochemical components identified in the methanol extract (ME), butanol (BU) and ethyl acetate (EA) fractions	132
Table 7.1	Dehydration scheme of fixed organs specimen	146
Table 7.2	Hematoxyllin & eosin staining procedure	147
Table 7.3	Effects of different extracts of *Ocimum tenuiflorum* L. on organ weights in STZ-induced treated rats	152
Table 7.4	Body weight of control and treated rats with limit test dose (2000 mg/kg) of hexane, chloroform, ethyl acetate, methanol and water extracts of *Ocimum tenuiflorum* L.during acute toxicity study	154
Table 7.5	Effects of different extracts of *Ocimum tenuiflorum* L. leaves on the plasma lipid profiles of STZ-induced diabetic rats	165
Table 7.6	Plasma urea, creatinine and bilirubin levels in control and STZ-induced diabetic rats with different *Ocimum tenuiflorum* L. leaves extracts	167
Table 7.7	Effects of different extracts of the leaves of *Ocimum tenuiflorum* L. on the plasma electrolyte profiles of STZ-induced treated rats	168

Table 7.8	Effects of different extracts of leaves from *Ocimum tenuiflorum* L. on the liver profile of STZ-induced treated rats	170
Table 7.9	Effects of different extracts on the leaves of *Ocimum tenuiflorum* L. on the haematological results of STZ-induced treated rats	173
Table 8.1	IC_{50} of acarbose, methanol crude extract, chloroform, ethyl acetate-butanol and ethanol-water fractions based on α-glucosidase inhibition assay	197
Table 8.2	IC_{50} of acarbose, methanol crude extract, chloroform, ethyl acetate-butanol and ethanol-water fractions based on α-amylase inhibition assay	200

LIST OF FIGURES

		Page
Figure 2.1	*Ocimum tenuiflorum* L. tree (Tulsi)	16
Figure 2.2	Flowers in elongate racemes in close whorls	16
Figure 2.3	Eugenol (1-hydroxy-2-methoxy-4-allylbenzene)	19
Figure 2.4	Urosolic acid (2,3,4,5,6,6a,7,8,8a,10,11,12,13,14 btetradecahydro-1H-picene-4a-carboxylic acid)	19
Figure 2.5	Carvacrol (5-isopropyl-2-methylphenol)	20
Figure 2.6	Linalool (3,7-dimethylocta-1,6-dien-3-ol)	20
Figure 2.7	Caryophylline (4,11,11-trimethyl-8-methylene-bicyclo [7.2.0] undec-4-ene)	20
Figure 2.8	Estragol (1-allyl-4-methoxybenzene)	20
Figure 2.9	Rosmarinic acid ((2R)-2-[[(2E)-3-(3,4-Dihydroxyphenyl) -1-oxo-2-propenyl]]oxy]-3-(3,4-dihydroxyphenyl) propanoic acid)	21
Figure 2.10	Apigenin(5,7-dihydroxy-2-(4-hydroxyphenyl) -4H-1-benzopyran-4-one)	21
Figure 2.11	Biosynthesis of phenolic compounds and praline-associated pentose phosphate pathway regulation in plants	35
Figure 2.12	The chemical structure of vitamin A	50
Figure 2.13	The chemical structure of vitamin E	51

Figure 2.14	The chemical structure of vitamin B1	53
Figure 2.15	The chemical structure of vitamin B3	54
Figure 2.16	The chemical structure of vitamin C	55
Figure 2.17	The chemical structure of lycopene	58
Figure 2.18	The chemical structure of β-carotene	58
Figure 3.1	Flow chart of the serial extraction of *Ocimum tenuiflorum* L. leaves	60
Figure 3.2	Flow chart of the liquid-liquid extraction of *Ocimum tenuiflorum* L. leaves methanolic extract	61
Figure 5.1	Effect of the extracts/metformin on blood glucose level after subcutaneous loading of 50 mg/kg	104
Figure 5.2	Effect of the extracts/ metformin on blood glucose level after subcutaneous loading STZ-induced diabetic rats	105
Figure 5.3	Effect of a single/acute dose of extracts/metformin on blood glucose of non-diabetic rats	107
Figure 5.4	Effect of a single / acute dose of extracts/metformin on blood glucose of STZ-induced diabetic rats	108
Figure 5.5	Effect of daily oral administration of extracts (1g/kg) or metformin (500 mg/kg) on blood glucose level of STZ-induced diabetic rats	110
Figure 5.6	Effect of oral administration of extracts (1g/kg) or metformin (500 mg/kg) on body weight (g) of	111

STZ-induced diabetic rats

Figure 5.7 Total phenolic content of *Ocimum tenuiflorum* L. leaves extracts 112

Figure 5.8 Total flavonoid content of *Ocimum tenuiflorum* L. leaves extracts 113

Figure 7.1 The steps of histopathology assessment, (A) Specimen collection and Preparation, (B&C) Specimen Preparation, (E) Hematoxyllin and Eosin staining procedure, (F) Studied under a light microscope 149

Figure 7.2 Microphotographs of kidney histology of different groups after treatments with *Ocimum tenuiflorum* L. leave extracts made with chloroform (OTCH), ethyl acetate (OTEE), methanol (OTME) and water (OTAQ) in STZ-induced rats (H&E staining10x) 156

Figure 7.3 Microphotographs of liver histology of different groups after treatments of *Ocimum tenuiflorum* L. leave extracts of chloroform (OTCH), ethyl acetate (OTEE), methanol (OTME) and aqueous (OTAQ) in STZ-induced rats (H&E staining 10x). CV: centralvenule, S: sinosides, SW: sinusoids widening 159

Figure 7.4 Microphotographs of pancreas histology of different groups after treatments of *Ocimum tenuiflorum* L. leave extracts of chloroform (OTCH), ethyl acetate (OTEE), methanol (OTME) 162

and aqueous (OTAQ) in STZ-induced rats (H&E staining 10x). IL: islet of langerhans, AC: acinar cells, MLD: mild langerhans disruption, AD: acinar disintegration, LD: langerhans disruption.

Figure 8.1 Insulin Assay Procedure 188

Figure 8.2 Effect of oral administration of different doses administration of active crude extract and metformin (500 mg/kg) on blood glucose level of STZ-induced diabetic rats 190

Figure 8.3 Effect of oral administration of different doses administration of active crude extract and metformin (500 mg/kg) on body weight (g) of STZ-induced diabetic rats 191

Figure 8.4 Effect of daily oral administration of fractions, metformin (500 mg/kg) on blood glucose level of STZ-induced diabetic rats 193

Figure 8.5 Effect of daily oral administration of fractions, metformin (500 mg/kg) on body weight of STZ-induced diabetic rat 194

Figure 8.6 Perecentage inhibition of yeast alpha glucosidase enzyme by methanol extract and its fractions of *Ocimum tenuiflorum* leaves and reference alpha glucosidase inhibitor, acarbose 196

Figure 8.7 Percentage inhibition of wheat alpha amylase enzyme by methanol extract and its fractions of *Ocimum tenuiflorum* L.leaves and reference alpha amylase inhibitor,acarbose 199

Figure 8.8 The effect of 14th day daily administration of *Ocimum tenuiflorum* L. methanol extract (ME) 1g/kg, chloroform, ethyl acetate-butanol, ethanol-water Fraction 500 mg/kg on insulin level of streptozotocin-induced diabetic rats 202

LIST OF ABBREVIATIONS

%	Percent
Cº	Degree centigrade
µg/mL	Microgram per milliliter
µl	Micro liter
Abs	Absorbance
ACR	Acarbose
ADI	Acceptable Daily Intake
ALT	Alanine Aminotransferase
ALP	Alkaline Phosphatase
AST	Aspartate Amino Transferase
ALK	Alkaline phosphatase
ANOVA	Analysis of Variance
AUC	Area Under Curve
B.W	Body Weight
BSA	Bovine Serum Albumin
BUF	n-Butanol Fraction
CCL4	Carbon Tetrachloride

CMC	Carboxymethyl Cellulose
CV	Coefficient of Variation
CHE	Chloroform Extract
CHF	Chloroform Fraction
DMSO	Di Methyl Sulph Oxide
DNS	Di Nitro Salicylic acid
DM	Diabetes Mellitus
DW	Distilled Water
EAE	Ethyl acetate Extract
ETF	Ethanol Fraction
EAF	Ethyl acetate Fraction
FFA	Free Fatty Acid
GAE	Gallic Acid Equivalent
GC-MS	Gas Chromatography Mass Spectroscopy
GLP1	Glucagon-Like Peptide-1
GLUT4	Glucose Transporter 4
GK	GlucoKinase
HbA	Hemoglobin A
HDL	High Density Lipoprotein

HeE	Hexane Extract
IgG	Immunoglobin G
IC50	Inhibition Concentration 50%
ip	intra peritoneal
IPGTT	Intra Peritoneal Glucose Tolerance Test
IR	Insulin Receptor
IRSs	Insulin Receptor Substrates
Kg	Kilogram
LD50	Lethal Dose 50%
µg	Microgram
NADH	Nicotinamide Adenine Dinucleotide
NIDDM	Non-Insulin-Dependent-Diabetes Mellitus
NO	Nitric Oxide
NC	Normal Control
mg	milligram
min	minute
mmol/l	milimol per litter
MeE	Methanol Extract
MeF	Methanol Fraction

OGTT	Oral Glucose Tolerance Test
OECD	Organisation for Economic Co-operation and Development
OT	*Ocimum tenuiflorum*
SEM	Standard Error of Mean
SD	Sprague Dawley
SPSS	Statistical Procedures for Social Sciences
STZ	Streptozotocin
SGTT	Subcutaneous Glucose Tolerance Test
Umol/l	Unit mol per litter
UV-VIS	Ultraviolet-Visible
v/v	Volume to volume
WHO	World Health Organisation
w/v	Water to volume
WE	Water Extract

PENILAIAN KESAN ANTIHIPERGLISEMIK, NILAI NUTRISI/ANTINUTRISI, DAN KANDUNGAN FITOKIMIA *OCIMUM TENUIFLORUM* LINN

ABSTRAK

Daun pokok *Ocimum tenuiflorum* L. (Lamiaceae) (dikenali sebagai ruku) telah digunakan untuk membuat nasi ulam di Malaysia. Oleh itu, penyelidikan ini dijalankan untuk meneroka kajian nutrisi, antinutrisi dan kesan antidiabetik pokok ruku. Hasil kajian fasa pertama mendapati daun pokok ruku mempunyai tinggi kandungan nurtisi (proksimat, profil asid amino, profil asid lemak, vitamin dan kandungan fitokimia) berbanding bahagian batang. Selain itu, keputusan paras antinutrisi dalam daun dan batang pokok ruku menunjukkan aras yang selamat. Oleh itu, dalam fasa kedua penyelidikan, daun diekstrak menggunakan pelarut yang berbeza untuk memilih ekstrak yang mengandungi kompaun dengan aktiviti antidiabetik yang tinggi dan menjalankan kajian akut, subakut toleransi glukosa dan ketoksikan subakut ekstrak dengan aktiviti yang terbaik. Ekstrak dengan aktiviti antidiabetik dibandingkan dengan kebolehan untuk merendahkan aras glukosa di dalam darah tikus normal dan tikus teraruh streptozotocin (STZ). Serbuk daun kering pokok ruku diekstrak dengan menggunakan heksana, kloroform, etil asetat, metanol dan air masing-masing menggunakan kaedah rendaman dan penyejat berputar. Keputusan menunjukkan bahawa ekstrak metanol mengurangkan aras glukosa puasa (FBG) dalam tikus diabetes teraruh streptozotocin. Kesan ekstrak etil asetat mengurangkan FBG lebih cepat berbanding dengan ekstrak lain; kadar pengurangan adalah 20% selepas 2 jam, 21% selepas 3 jam dan 8% selepas 5 dan 7 jam. Selepas 7 jam, kesan ekstrak metanol ke atas FBG adalah lebih rendah secara signifikan

daripada metformin. Di dalam ujian toleransi glukosa subkutaneus, hanya ekstrak methanol dan heksana sahaja menunjukkan persamaan metformin di dalam tikus diabetes. Pengambilan ekstrak secara oral pada tikus teraruh STZ menunjukkan bahawa selepas 14 hari, kesan ekstrak adalah sama dengan metformin (63.33%). Kajian hispatologi pada vital penting menunjukkan morfologi yang normal dalam hati, buah pinggang dan sel pankreas dalam kumpulan rawatan diabetes apabila dibandingkan dengan kumpulan kawalan, jadi kajian menunjukkan bahawa ekstrak tiada kesan toksik. Tambahan pula, penyiasatan antihiperglisemik pada dos yang berbeza (500, 250, 125 mg/kg bw) ekstrak mentah yang aktif (metanol) dalam tikus teraruh-STZ menunjukkan bahawa ekstrak methanol dos 500 mg/kg bw mempunyai kebolehan paling tinggi untuk mengurangkan aras glukosa dalam darah di dalam ujian akut. Di dalam fasa ketiga penyelidikan, aktiviti antihiperglisemik pecahan metanol kemudiannya dinilai secara akut, pengulangan pengambilan ekstrak klorofom 500 mg/kg secara oral, etil asetat-butanol dan pecahan etanol-akuas selama 14 hari. Hasilnya menunjukkan bahawa pecahan etil asetat-butanol mempunyai kesan antihiperglisemik yang sangat tinggi berbanding dengan pecahan kloroform dan etanol-air. Selain itu, kesan ekstrak aktif dan pecahannya terhadap aktiviti enzim α-glucosidase dan α-amylase dan aras insulin (*in-vitro*), pecahan etil asetat-butanol boleh mengawal kepekatan yang rendah berbanding dengan pecahan yang lain dan akarbos digunakan sebagai kawalan positif. Keputusan insulin etil asetat-butanol, menunjukkan ares lebih tinggi berbanding dengan pecahan lain dan metformin mungkin disebabkan oleh kebolehan untuk tidak merosakkan sel-β untuk menghasilkan insulin yang mencukupi. Dapatan kajian juga mendapati ekstrak mentah yang aktif (metanol) dan pecahan aktifnya (etil asetat, butanol) boleh menghasilkan kesan pengurangan glukosa yang signifikan kerana hadirnya polifenol

yang aktif seperti asid 3,4-dimethoxycinnamic, asid kafeik, diosmetin, luteolin, kaempferol, asid rosmarinic, apigenin and genistein. Kesimpulannya, daripada keputusan nilai nutrisi dan anti-nutrisi daun *Ocimum tenuiflorum* dan ekstrak metanol dan fraksinya, ia adalah agen yang baik dalam industri nutraseutikal dan makanan. Tambahan pula, pengasingan komponen aktif boleh digunakan ke arah kaedah baru untuk rawatan diabetes dan komplikasinya.

EVALUATION OF ANTIHYPERGLYCEMIC EFFECTS, NUTRITIONAL/ANTINUTRITIONAL VALUES, AND PHYTOCHEMICAL CONTENTS OF *OCIMUM TENUIFLORUM* LINN

ABSTRACT

The leaves of *Ocimum tenuiflorum* L. (lamiaceae) (locally known as *ruku*) has been used to make nasi ulam in Malaysia. Therefore, the present work is aimed to explore the nutritional, antinutritional, and it`s anti diabetic effect on *ruku* tree. The result of the first phase showed that *ruku* leaves displayed higher values of the nutritional level (proximate, amino acid profile, fatty acid profile, vitamin and phytochemical content) compared to the stem. In addition, the result of antinutrients level in the *ruku* leaves and stem displayed within the safe level. Therefore, in the second phase of the study, the leaves were extracted using different solvents to select the extract that contains compound(s) with the strongest anti-diabetic activity and to carry out acute, subcutaneous glucose tolerance, and sub-acute toxicity tests of the extracts with the best activity. The anti-diabetic activity of the extracts was compared based on their ability to reduce the blood glucose level of normal and streptozotocin-induced rats. Dried powdered leaves of *ruku* were extracted with hexane, chloroform, ethyl acetate, methanol and water consequently by maceration method and concentrated using rotary evaporator respectively. The result showed that the methanol extracts reduced the fasting blood glucose of the streptozotocin-induced (STZ) diabetic rats. The FBG-decreasing effect of ethyl acetate extract was more rapid than that of the other extracts. The decreasing rates were 20% after 2h, 21% after 3h, and 8% after 5 and 7h. After 7h, the effect of methanol extract on FBG was significantly lower than metformin. In the subcutaneous glucose tolerance test,

only methanol and hexane extracts showed the similar of metformin in diabetic rats. Daily oral administration of extracts in STZ-induced rats showed that after 14 days, the effects of these extracts were similar to those of metformin (63.33%). The histopathology study of vital organs presented normal morphology in liver, kidney and pancreas cells in diabetic treatment groups compared to control group, suggesting no toxic effect of extracts. In addition, investigation of anti hyperglycaemic of oral administration of different dose (500, 250, 125 mg/kg bw) by active crude extract (methanol) in STZ-induced rats showed that 500 mg/kg bw the dose of methanol extract with the highest lowering blood glucose level in the acute test. In the third phase of the study, antihyperglycemic activity of methanol fraction was then assessed by acute, repeated administration of 500 mg/kg of chloroform, ethyl acetate-butanol and ethanol-water fractions for 14 days. The result indicates that ethyl acetate-butanol fraction exhibited a stronger antihyperglycemic effect than chloroform and ethanol-water fractions. Moreover, the result showed that effect of active extract and its fraction on α-glucosidase and α-amylase enzymes activities and its insulin level (*in vitro*), ethyl acetate-butanol fraction could control with low concentration compared to other fractions and acarbose that used as a positive control. From the insulin level result only normal group showed the significant difference compared to diabetic group and other extract and fraction did not showed any significant. These findings showed that the active crude extract (methanol) and its active fractions (ethyl acetate, butanol) could exert a significant glucose lowering effect due to the presence of polyphenolics active constituents such as 3,4-dimetthoxycinnamic acid, caffeic acid, diosmetin, luteolin, kaempferol, rosmarinic acids, apigenin and genistein. In counclusion, form the result of nutritional and anti-nutritional values of *Ocimum tenuiflorum* leaves and its methanol extract and

fractions, it is a good agent in nutraceutical and food industries. Furthermore, isolation of the active components may pave the way to the development of new agents for the treatment of diabetes and its complications.

CHAPTER 1

INTRODUCTION

1.1 Background

Being one of the types of endocrine metabolic disorders, diabetes mellitus (DM) or hyperglycemia is not uncommon. Due to its micro vascular and macro-vascular complications, morbidity and mortality are the outcomes of this disorder (Patel *et al.*, 2012). Diabetes comprises of type I and type II. Type I is known as insulin-dependent diabetes mellitus (IDDM). It is the source of 5% to 10% of diabetes cases, where any insulin could not be produced from the body. Therefore, daily insulin injection is required for the life of individuals with type I diabetes (Patel *et al*., 2012). Meanwhile, type II is known as non-insulin-dependent diabetes mellitus and 90% to 95% of diabetes cases are the result of this type of diabetes mellitus. In this type of diabetes, the body has been fails in the production or proper use of insulin properly (Li *et al*., 2004). As proven by the World Health Organization (WHO, 2010), there has been an outstanding increase in diabetes mellitus over the past two decades. To illustrate this, current trends have shown that there will be 360 million of diabetes patients by year 2030 (Letchuman *et al*., 2010).

Considering that prevalence studies have only been conducted in 91 countries, Middle-East is the country with 5 of the 10 world's highest national prevalence (Table 1.1), although only Saudi Arabia (16.8%) is one of the 80 countries with the highest population. (Amos *et al.*, 1997).

Table 1.1 Top 10 countries for diabetes prevalence in 2010 and 2030

	2010		2030	
	Country	Prevalence (%)	Country	Prevalence (%)
1	Nauru	30.9	Nauru	33.4
2	United Arab	18.7	United Arab	21.4
3	Saudi Arabic	16.8	Mauritius	19.8
4	Mauritius	16.2	Saudi Arabia	18.9
5	Bahrain	15.4	Reunion	18.1
6	Reunion	15.3	Bahrain	17.3
7	Kuwait	14.6	Kuwait	16.9
8	Oman	13.4	Toga	15.7
9	Toga	13.4	Oman	14.9
10	Malaysia	11.6	Malaysia	13.8

Only includes countries where survey with blood glucose testing were undertaken for that country (Letchuman *et al*., 2010).

Additionally, Malaysia population has high risk of the epidemic of diabetes mellitus compared to the population in other developing countries. For this matter, the global trend, socioeconomic transition, and common risk factors of the disease are put into account (Yun *et al*., 2007). Therefore, diabetes is a major health concern among the public in Malaysia. Similar to the premature and preventable mortality, there is a close relation between this disease to the rising number of macro- and micro-vascular problems, (Letchuman *et al*., 2010). There had been the increase of the prevalence of type II diabetes (T2D) among adults aged 30 years and older in Malaysia over the previous decades (Mohamed 2008). Based on the report made by the fourth Malaysian National Health and Morbidity Survey (NHMSIV) in 2011, where the prevalence amounted to 14.9% in 2006, there was an increase in the prevalence of T2D by 20.8%. Compared to the prevalence of this diabetes reported by the third National Health and Morbidity Survey (NHMS III), 2.8 million individuals were significantly affected (Letchuman *et al*., 2010).

Moreover, Indians have the highest prevalence of T2D, which was 24.9% in 2011 and 19.9% in 2006 Malays came in second, where the prevalence was 6.9% in 2011 and 11.9% in 2006, followed by Chinese, with 13.8% of prevalence in 2011 and 11.4% in 2006 (Mafauzy *et al*., 2011; Mohamed, 2008). Apart from that, Malaysians got worse when it comes to glycaemic control with T2D continued with the rise of the mean haemoglobin A1c in terms of percentage, which is (A1c) from 8.0% in 2003 to 8.66% in 2008 (Mafauzy *et al*., 2011; Mohamed, 2008).

Based on the data shown from the online registry database of Adult Diabetes Control and Management, glycemic control is influenced by differences in ethnicity. This can be seen from how the ethnicity in Malaysia with the lowest mean of A1c levels (7.8%) is Chinese with T2D, the ethnicity with the highest mean, which was

8.5%, is Asian Indians (Chew *et al*., 2011). The parallel rise in the prevalence of overweight and obesity is the remarkable result of the prevalence of T2D. It was reported that the overall prevalence of obesity in Malaysia ranged from 55.6% to 57.4%, based on the individuals' waist circumference (Mohamud *et al*., 2012; Zaki *et al*., 2010). Besides, Malaysia has consistently proven that there is an ethnic trend which is similar to the one observed in T2D when it comes to obesity. This could be seen from Asian Indians displaying the highest prevalence, which is from 65.5% to 68.8%. This is followed by Malays, with prevalence of 55.1% to 60.6%, Chinese with prevalence of 49.5% to 51.1%, and other indigenous groups, where their prevalence was from 44.9% to 48.3% (Mohamud *et al*., 2012; Zaki *et al*., 2010). Due to the increasing prevalence of abdominal obesity in people with T2D, obesity and diabetes are connected to each other. It was found that 75% of Malaysians with T2D were obese (Mafauzy *et al*., 2011). Additionally, based on the report shown from Malaysia Diabetic Care study in 2008 more women had undesirable waist circumference (≥80 cm in 89.4%) compared to men (≥90 cm in 73.7%) with T2D (Mafauzy *et al*., 2011). Apart from that, it was also found that from 72% of people with T2D had a mean body mass index (BMI) of 27.8 kg/m^2, and they were patients with obesity (Mafauzy *et al*., 2011). Furthermore, it was stated in the National Obstetric Registry 2nd Report diabetic pregnant women encompass 9.90% of diabetic patients in 2010 (Hussein *et al*., 2015). To illustrate this, most of them were diagnosed with gestational diabetes (11,848 [8.66%]), whereas 1009 (0.74%) of them were diagnosed with presentational diabetes (Hussein *et al*., 2015). The Asian Indians (14.39%) had the highest occurrence of diabetes in pregnancy, while the Malays (11.37%) and Chinese (10.4%) came in second. Most of them were 31 and 40 years old (48.3%) (Hussein *et al*., 2015). Not only that, the rate of caesarean

section was higher (14.7%) among pregnant women with diabetes, compared with vaginal deliveries (8.5%) (Hussein *et al*., 2015). It was also known that Type 1 diabetes (T1D) is dominant among children and adolescents through the observation that 71.8% of patients (Hussein *et al*., 2015) were diagnosed with it.

Besides, the median age of this group of people were 7.6 (inter quartile range: 4.6, 10.8) years old, 3.3 years of diabetes (Hussein *et al*., 2015). On the other hand, not only most (42.3%) of the patients with T1D were from 10 to 15 years old, 57.5% of them are diagnosed with diabetic ketoacidosis. T1D or T2D was discovered in a positive family history, where 50.2% of them among patients themselves (Hussein *et al*., 2015). Over weight is the problem faced by approximately 11.8% of patients with T1D (Hussein *et al*., 2015). However, T1D occurred in only 0.6% of adults (Hussein *et al*., 2015). Furthermore, food which provide health benefits and prevent various types of diseases is reported by Stephaen Defeliece in 1989 (Kalra, 2003). It was also pointed out by Hippocrates that food is useful for treating different types of diseases. He also stated "*Let food be thy medicine and medicine be thy food*" (Bernal *et al*., 2011). Recently, the fact that there is a significant correlation between an individual's health condition and his/her good dietary routine is believed. Besides, American Dietetic Association (ADA) in 2004 emphasized that positive health depends on what is provided in a particular dietary (Kant, 2004). The emphasis on the importance of dietary intake has also become stronger due to the principal changes occurred in human-health, added with the consideration of new methods when it comes to nutrition (Biesalski *et al*., 2009). However, the fact that whether the biological aspects and structural variegation of small molecule-secondary metabolites are originated from nature are still unclear (Bradacs *et al*., 2010; Khanna *et al*., 1979). There has been an increase in the number of studies which explore on

the significant role of biodiversity in enhancing health condition (Bradacs *et al*., 2010). It is an aromatic plant, known as Holy Basil, *tulsi*, or *tulasi* worldwide. Furthermore, nasi ulam, a famous local dish in Malaysia especially in Perak state, involves the use of the leaves for the making of it (Khanna *et al*., 1979). In fact, there are useful advantages to all parts of *Ocimum tenuiflorum*, especially for medical treatments. They have also been utilized for folk medicines and the treatment to different diseases (Cox, 1967).

1.2 Aims and Objective

Traditional medicinal plants species are originated from ancient times. Based on the estimation by WHO, up to 80% of plants are utilized in most developing countries (Kayarohanam & Kavimani 2015). Furthermore, almost 420,000, 248,000 number of plant species has been identified in nature, and medicinal properties were discovered in 12,000 plants. However, investigation from a phytochemical or pharmacological point of view with bioactivities was conducted on less than 10% of all the plants (Husain Shahnaz *et al*., 2011). On the other hand, medicinal plants are proven to have more benefits than conventional drugs, due to the test conducted on their long term use by humans in various forkloric usages (Husain Shahnaz, *et al*., 2011). Therefore, it can be predicted that there is a low level of human toxicity in any bioactive compounds based on medicinal systems from the past. A part from that, on the physiological aspect, the absorption of natural products with higher molecular weights and rotatable bonds with stereo genic centres is higher than synthetic drugs (Ntie-Kang *et al*., 2013). Therefore, the use of bioactive compounds, which are isolated from the active extracts of the plants utilized in traditional medicines, is possible for the development of novel drugs. This development is conducted through the method of semi-synthesizing along the lead compounds, in

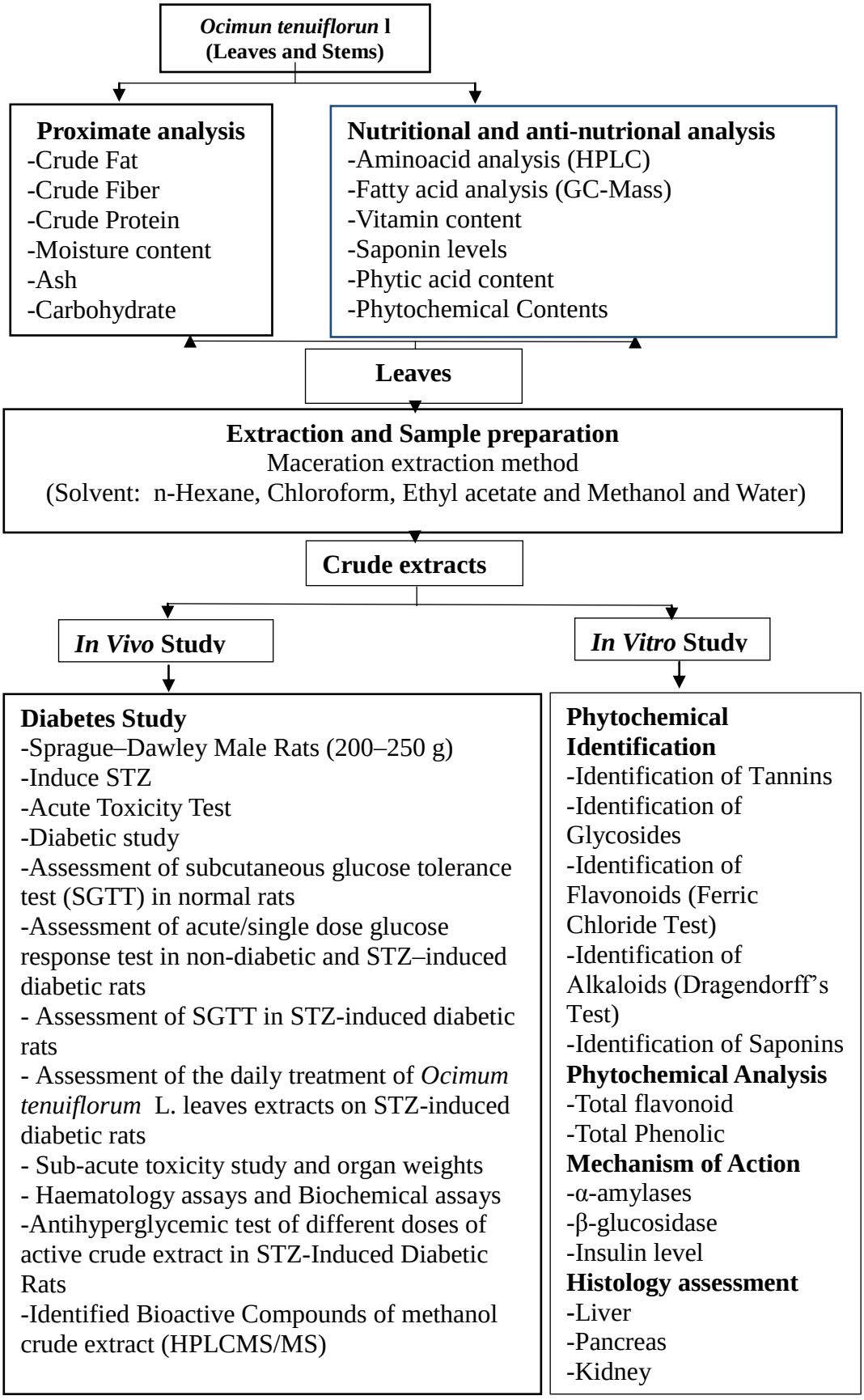
order to produce higher activity and/or lower toxicity rate. As of recently, the treatment for diseases relating to healthy food supplements instead of the use of other kind of chemical treatment is increasingly demanded. The same case has occurred to hyperglycaemic treatment. However although the benefits of the *Ocimum tenuiflorum* L. against diabetic disease is numerous, there is a scarce amount of information about it in Malaysia. This can be seen from the absence of study conducted in Malaysia, which objectively investigates the hyperglycaemic and antihyperglycemic of crude extracts in food, such as the leaves of *Ocimum tenuiflorum* L. This finding could provide benefits for further studies conducted on these samples for *in vivo* study from hyperglycaemic. This is provided if lower blood glucose level is seen from the crude extracts.

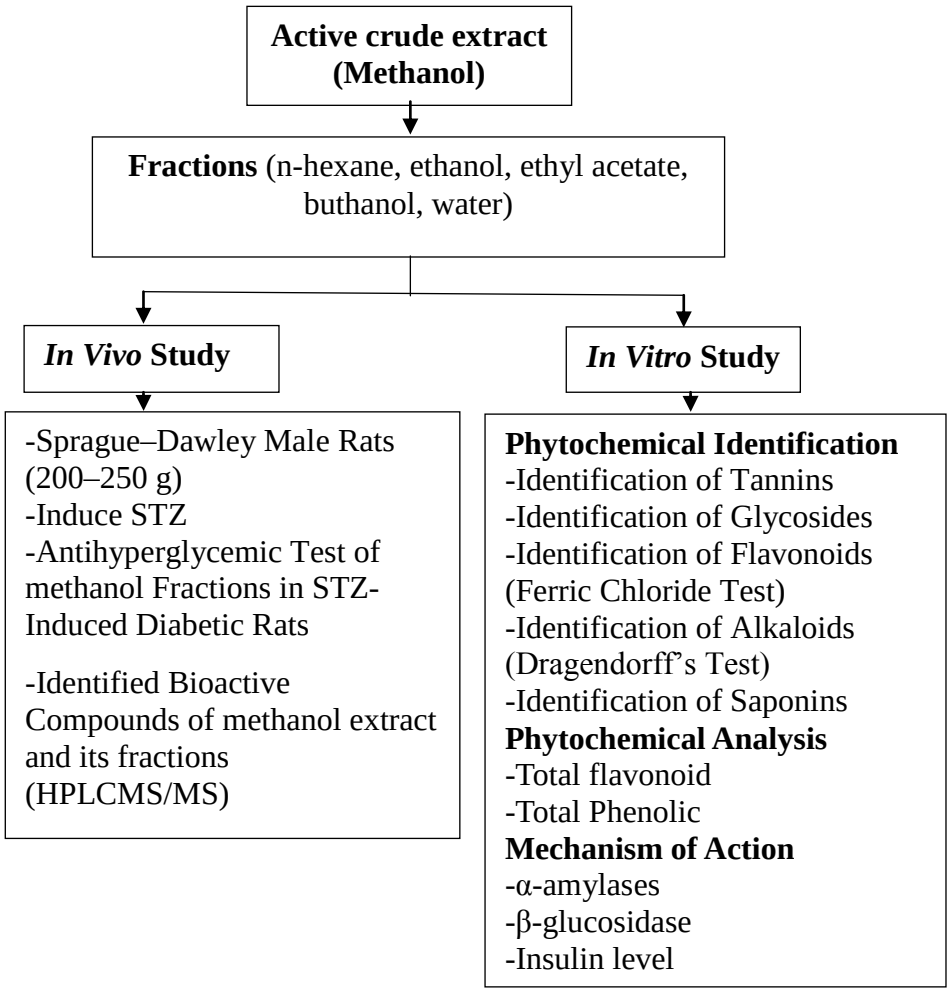
The main objective of this study is to determine the nutritional, antinutritional composition and antihyperglycemic activities by *in vitro* and *in vivo* studies. The specific objectives of the research were:

1. To determine the nutritional and anti-nutritional values from the leaves and stems of *Ocimum tenuiflorum* L.
2. To evaluate the antihyperglycaemic activities of differents extracts of *Ocimum tenuiflorum* L. leaves extraction by hexane, chloroform, ethyl acetate, methanol and water on normal and STZ-induced diabetic rats.
3. To fractionate the most active antihyperglycemic crude extract and investigate the antidiabetic activity for fraction in the normal and STZ-induced diabetic rats.
4. To determine toxicity effect of the extracts on vital organs (histopathology assessment of kidney, liver and pancreas).

5. To study the effect of active crude extract and fraction on alpha amylase and alpha glucosidase inhibitions *in vitro* and determine its half maximal inhibitory concentrations IC_{50}.
6. To study the effects of active crude extract/fraction by *in vitro* insulin inhibition and determine its $IC_{50.}$

1.4 Conceptual frame work




Active crude extract (Methanol)
Fractions (n-hexane, ethanol, ethyl acetate, buthanol, water)
In Vivo Study
In Vitro Study
-Sprague–Dawley Male Rats (200–250 g)
-Induce STZ
-Antihyperglycemic Test of methanol Fractions in STZ-Induced Diabetic Rats
-Identified Bioactive Compounds of methanol extract and its fractions (HPLCMS/MS)
Phytochemical Identification
-Identification of Tannins
-Identification of Glycosides
-Identification of Flavonoids (Ferric Chloride Test)
-Identification of Alkaloids (Dragendorff's Test)
-Identification of Saponins
Phytochemical Analysis
-Total flavonoid
-Total Phenolic
Mechanism of Action
-α-amylases
-β-glucosidase
-Insulin level

CHAPTER 2

LITERATURE REVIEW

2.1 Medicinal Plants

Plants have been a source of potency required for phytomedicines since long time ago. Various industrial chemicals could be formulated from them. Furthermore, Atharvaveda (an Indian religious book), includes ancient scholastic work known as the Ayurveda (an Indian system of traditional medicine), and others (Cragg & Newman, 2001), are the heritage where rich information regarding preventive and curative medicines are stored. It is estimated that drugs are known to be created with approximately 13000 species of plant in the world (Oloyede *et al*., 2015). Moreover, the seeds, fruits, roots, flowers, leaves, bark, and any parts of the trees, can be made into plant-based natural constituents (Cragg & Newman, 2001), provided if there is any active component within any part of the plant. Generally, the positive medicinal outcomes of plant materials stem from the combination of the secondary products existing within the plant (Cox, 1967). The fact that each particular plant species or group having its own unique medicinal actions, is consistent with this concept. This is because each plant's combination of secondary products taxonomically distinguish themselves from one another (Oloyede *et al*., 2015). In many laboratories, in order to discover new bioactive compounds, the systematic screening conducted on plant species in search is a routine activity. An extended research regarding medicinal plants should be conducted, and the active principles in the plants should be identified. Scientific investigation should be performed for the products safety, as it will result in the standardization and quality control (Cragg & Newman, 2001). Following that, these products could be certified for primary health care uses.

Besides, the development of new drugs could be performed through these research activities, as in the past (Cox, 1967).

Additionally, the basis for the creation of new life-saving drugs is the result of the vitammin exploration conducted on the chemical contents existing in plants and pharmacological screening. Following that, members of the Lamiaceae family are the medical plants frequently used to preserve and add flavours into food, and to treat common illnesses (Shetty, 1997). Furthermore, there are the contents of phenolic phytochemicals in these herbs, which are important for human health (Lade *et al*., 2012). This indicates that hypoglycaemic effects possibly come from members under the Lamiaceae family, where phenolic contents such as rosmarinic acid exist. The high rate of α-glucosidase inhibitory activities are the antecedent of this. Following that, for both the aqueous and ethanolic extracts, the inverse correlation ($r = -1$) between antioxidant activity and α-glucosidase inhibition were observed on respectively. Based on this observation, it is possible that the inhibitory activity of α-glucosidase will not be affected by the antioxidant activity (Lade *et al*., 2012), in spite of the high percentage of DPPH in aqueous and ethanolic extracts (Siddiqui & Prasad, 2017). Besides, it has been suggested from the results from the study conducted by (Siddiqui & Prasad, 2017) that the dietary enrichment of Lamiaceae herbs could enhance health, when they are converted into condiments or other consumables. This can be done by enriching the functional phenolic bioactive contents within food. Not only that, due to high rate of the activity of α-glucosidase inhibitory, blood glucose levels could be reduced and the duration of carbohydrate absorption could be extended, provided if they are consumed in our diet (Lade *et al*., 2012). Meanwhile, as the particular phenolic phytochemicals are not present, α-amylase inhibition was investigated for the extracts of ethanolic or aqueous extracts,

which are present in marjoram and sage. The inhibition of α-amylase is the outcome of this. Despite the absence of α-amylase inhibition in observation, through the inhibition of high α-glucosidase it can be seen that it is still useful as food ingredient and condiment (Siddiqui & Prasad, 2017).

This could target the treatment of hyperglycaemia, which has a connection with type 2 diabetes. Other than that, the absence of Angiotensin Converting Enzyme (ACE) inhibition was observed. This implies that other phenolic compounds or peptides were present. Lamiaceae plant extracts, which have connection with ACE inhibition, are abundant in these chemicals (Kwon *et al*., 2006). Based on the phenolic phytochemicals analysis conducted on high-performance liquid chromatography (HPLC), rutin, rosmarinic acid and caffeic acid are three major 9 phenolic compounds which could be found in both aqueous and ethanolic extracts (Kwon *et al*., 2006). To illustrate this, the content of rosmarinic acid in aqueous extracts is higher than the content of rosmarinic acid in ethanolic extracts. Therefore, the amount of α–glucosidase inhibitory activity for aqueous extracts was higher compared to that for ethanolic extracts (Siddiqui & Prasad, 2017).

The plants of the *Ocimum* genus are a very important member of the Lamiaceae family for their therapeutic potentials. They come from a species of plants that are known for their medicial value. Furthermore, *Ocimum tenuiflorum* consists of two types: black *(Krishna Tulsi)* and green *(Rama Tulsi)* with similar chemical constituents (Warrior, 1995). Tulsi is a Sanskrit word which means "matchless one". In fact, a number of medicinal properties are created from the Tulsi plant, not only in Ayurveda and Siddha but also in the Greek, Roman, and Unani medicinal systems (Cox, 1967). However, the plants were used for medical purposes long time ago in the past. This can be seen from literature's indication that the therapeutic use of

plants had begun since 4000-5000 B.C. Additionally, the preparation for the first natural herbal were utilized by the Chinese as medicines (Warrior, 1995). Rigveda contains the earliest references regarding these plants, which dates between 3500-1600 B.C. (Biswas & Biswas, 2005). It should be noted that, out of the various healing and health-giving herbs mainly distributed in the oriental region, the holiest and appreciated herbs are the Legendary, "Incomparable One", Queen of Herbs, and Tulsi (*Ocimum sanctum*), (Sen, 1993). To illustrate this, Tulsi a well known, sacred plant which is a member of the Lamiaceae family. There are many alternative names for it, such as Rama Tulsi Krishna Tulsi (Sanskrit) and holy basil (English).

An altitude of 200 m of sea level is the range for the natural habitat of Tulsi. Moist soil in almost all parts of the globe is the area where it usually grows (Gupta *et al*., 2004). Rama tulsi, Krishna tulsi, and Vana Tulsi are the three main forms of this plant which are acknowledged. Rama tulsi is the type with green stems and leaves, while Krishna tulsi consists of purple stems and sometimes purple leaves.

Meanwhile, Vana tulsi retains its wild form. Furthermore, the differences between the sizes, forms of the plants, and their medicinal strength and efficacy are represented by the differences between soil types and rainfall. Moreover, the *Ocimum* genus contains species of herbs and shrubs from the tropical regions of Asia, which range between 50 and 150 of them (Chopra *et al*., 1956). Following that, the plants come with square stems, whorled flowers on spiked inflorescences, and fragrant opposite leaves (Anbarasu & Vijayalakshmi, 2007). Basil essential oil, which is extracted via steam distribution from the leaves and flavouring tops, are utilised intraditional remedies and medicines, , dental and oral products, fragrances, and food flavouring (Kelm *et al*., 2000). It has been proven that biologically active constituents are present in extracted essential oils, and they contain are insecticidal

(Jaggi & Low, 2000), nematocidal (Aina *et al*., 2012), and fungistatic (Narendhirakannan & Kandaswamy, 2006). These properties are mostly like due to predominate essential oil constituents, such as methyl cinnamate, camphor, eugenol linalool, andmethyl chavicol. It has been reported that two minor components of the sweet basil essential oil (*Ocimum basilicum*) - Juvocimene I and II - are dominant juvenile hormone analogues (Hannan *et al*., 2006).

2.2 Botany of *Ocimum tenuiflorum*

Kingdom: Plantae

(unranked) Angiosperms

(unranked) Eudicots

(unranked) Asterids

Order: Lamiales

Family: Lamiaceae

Genus: *Ocimum*

Species: *Ocimum tenuiflorum*

Binomial name: *Ocimum tenuiflorum* or *Ocimum tenuiflorum* L.

One of the features of *Ocimum tenuiflorum* L. (Tulsi) is its strong scent. It comes in an erect, 30-60 cm tall branched sub shrub, hairy stems, and constrasting green or purple leaves (Figure 2.1).

Figure 2. 1 *Ocimum tenuiflorum* tree (Tulsi)

Besides having petiole, the leaves have somewhat toothy edges, and their oval shapes can go up to 5 cm long. Furthermore, the flowers have purple shades, and come with elongated racemes in close whorls (Figure 2.2).

Figure 2. 2 Flowers in elongate racemes in close whorls

Within the tropics around the world, Tulsi is native and ubiquitous as a cultivated plant and a preserved weed. The plantation of it is for religious and medicinal purposes. Besides, essential oil is also extracted from this plant. Moreover, Tulsi is a

significant symbol in many Hindu religious traditions, where a connection exists between the plant and Goddess figure. In Sanksrit, the name of this plant means 'the incomparable one'. The religious bend of a Hindu family is presented by the appearance of Tulsi (Chopra *et al*., 1956; Sen, 1993).

Following that, due to the various healing properties, the use of *Ocimum tenuiflorum* L. (also known as *Ocimum tenuiflorum*, Tulsi) has been going on for thousands of years in Ayurveda. *Ocimum micranthum*, *Ocimum camphora*, *Ocimum americanum*, *Ocimum kilimandschricum*, *Ocimum bascilicum* (Ban Tulsi), *Ocimum canum* (Dulal Tulsi), and *Ocimum gratissimum* (Ram Tulsi), are examples of the primary species of genus *Ocimum*. Besides growing in various parts of the world, they are renowned for their medicinal properties (Chopra *et al*., 1956; Sen, 1993).

2.2.1 Micronutrient status in diabetes mellitus

In addition, as early as 1674, patients with diabetes mellitus were recommended by Sir Thomas Willis that they should have gummy and starchy food. Based on this incident, identification was performed on the complex relationship between nutrition and diabetes mellitus (Mooradian & Morley, 1987). Since the last 20 years, numerous studies have found the alterations in micronutrient status of patients of diabetes mellitus . Not only that, there has been a correlation between the inadequacy of particular types of minerals or vitamins and the presence of diabetic complications (Nuttall, 1983). A number of studies were conducted regarding the vitamin and mineral composition within patients of diabetes mellitus and the possible roles of these substances in pathogenesis of diabetes mellitus. These studies are summarized in Table 2.1 (Anderson *et al*., 1980; Vinik & Jenkins, 1988). Based on the what is presented in Table 2.1 there was a significant decrease in the amount of minerals, vitamin B12, and vitamin C formed by zinc and magnesium within both type I and II

diabetes. Meanwhile, different impacts were displayed by the other minerals and vitamins. This could be seen from the decrease of the diabetic level two types of japanese diabetes patients, which are Type I and Type II.

Table 2. 1 Micronutrient status of diabetic patients.

	Status in diabetic patients	
Micronutrient	Type I	Type II
I TRACE ELEMENTS		
Zinc (Zn)	↓	↓
Chromium (Cr)	↑	NL*
Calcium (Ca)	↓	NL
Magnesium (Mg)	↓	↓
Copper (Cu)	NL	NL or ↑
Manganese (Mn)	↓ or NL	↑↑
Iron (Fe)	NL	NL
Selenium (Se)	↑	?
II Vitamis		
A	.....?	NL
Thiamin	NL	NL↑
B-6	NL or ↓	NL or ↓
B-12	NL or ↓	NL
C	NL or ↓	NL or ↓
1,25-dihydroxycholecaliferol	↓	NL
E	↑	↑

*NL= neither increased or decreased.

2.2.2 Phytochemicals and Bioactivecompounds constituents

Due to the abundance of nutrients and other biologically active compounds present in Tulsi, its chemical is significantly complex. To illustrate this, there are the presence of eugenol (1-hydroxy-2-methoxy-4-allylbenzene (Figure 2.3), euginal (also called eugenic acid), urosolic acid (Shishodia, 2003) (2,3,4,5,6,6a,7,8,8a,,10,11,12,13,14b-tetradecahydro-1H-picene-4a-carboxylic acid (Figure 2.4), carvacrol (5-isopropyl-2-methylphenol (Figure 2.5), linalool (3,7-dimethylocta-1,6-dien-3-ol (Figure 2.6), limatrol, and caryophyllene (4,11,11-trimethyl-8-methylene-bicyclo (7.2.0). This is followed by undec-4-ene (Figure 2.7),

and methyl carvicol (also called Estragol: 1-allyl-4-methoxybenzene (Figure 2.8). They are all present within the leaf volatile oil (Kelm *et al*., 2000). On the other hand, fatty acids and sitosterol are contained in the seed volative oil. In addition, there is a content of some levels of sugar within the seed mucilage, along with the presence of anthocyans in green leaves.

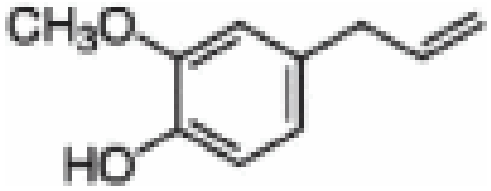


Figure 2. 3 Eugenol (1-hydroxy-2-methoxy-4-allylbenzene)

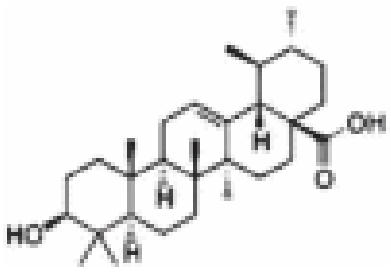


Figure 2. 4 Urosolic acid (2,3,4,5,6,6a,7,8,8a,10,11,12,13,14btetradecahydro-1H-picene-4a-carboxylic acid)

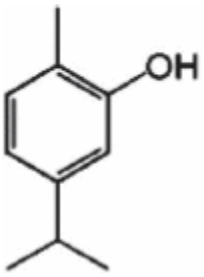


Figure 2. 5 Carvacrol (5-isopropyl-2-methylphenol)

Figure 2. 6 Linalool (3,7-dimethylocta-1,6-dien-3-ol)

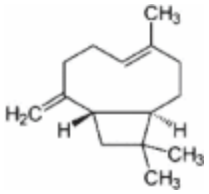


Figure 2. 7 Caryophylline (4,11,11-trimethyl-8-methylene-bicyclo [7.2.0] undec-4-ene)

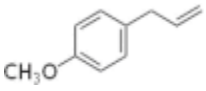


Figure 2. 8 Estragol (1-allyl-4-methoxybenzene)

Additionally, there are contents of xylose and polysaccharides in the sugar (George, 2011). However, caffeine or other stimulants are not present in it, although it is renowned as a general vitalizer which improves physical endurance (Kelm *et al*., 2000). In addition, there are various constituents within the holy basil's stem and leaves. which may undergo biological activity, including tannins, triterpenoids, flavonoids, and saponins (Jaggi & Low, 2000). Besides, it has been identified that antioxidant and antiinflammatory activities take place in the following phenolic actives. Examples of the activities are isothymusin (6,7-dimethoxy-5,8,4'-trihydroxyflavone) cirsimaritin (5,4'-dihydroxy-6,7-dimethoxyflavone), rosmarinic acid ((2*R*)-2-[[(2*E*)-3-(3,4-Dihydroxyphenyl)-1-oxo-2-propenyl]]oxy]-3-(3,4-dihydroxyphenyl), propanoic acid (Figure 2.9), apigenin (5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one (Figure 2.10), and isothymonin. It has been proven by two water-soluble flavonoids: orientin (8-C-beta-glucopyranosyl-

3’,4’,5,7-tetrahydroxyflav-2-en-3-one) and vicenin (6-C-beta-D-xylopyranosyl-8-C-beta-D-glucopyranosyl apigenin), that they protect human blood lymphocytes from radiation-induced chromosomal damage.

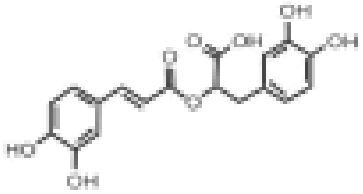


Figure 2. 9 Rosmarinic acid ((2R)-2-[[(2E)-3-(3,4-Dihydroxyphenyl)-1-oxo-2-propenyl]]oxy]-3-(3,4-dihydroxyphenyl)propanoic acid)

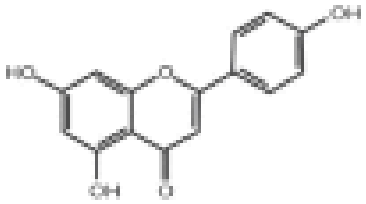


Figure 2. 10 Apigenin (5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one)

2.3 Cytotoxic effects of medicinal plant

There are numerous active ingredients within plants that can result to negative impacts due to improper use of medicinal herbs. Besides, there is a frequent occurrence of drug-plant interactions. Despite this fact, usually the subtle side impacts of most medicinal herbs are not known. However, physicians should have an awareness regarding the possibility of a patient suffering from the negative impacts of medicinal herbal remedy. Nevertheless, herbal remedies can act either as agonists or antagonists that enhance several drug therapies. Therefore, the understanding regarding conventional drugs is necessary for effective herbal therapeutics (George, 2011). Scientists have been able to detect each little amount of carcinogenic and toxic chemicals within these herbs due to technological advancement. Besides, they have been able to recognize or evaluate potentially dangerous impacts of some of the herbs that have been used for traditional remedies since centuries ago (Kelm *et al*.,

2000). In fact, there are numerous herbs with contents of nearly pharmaceutical concentrations of poisonous constituents, which, by any means, should not be consumed internally by unqualified persons other than people with homeopathic tendencies. The limitation of the availability of these herbs is reinforced by law. Besides, herbs with significant effects often result to nausea or vomiting, (which are traditionally prized for this action). Nevertheless, the use of these herbs are perfectly safe under appropriate conditions. Even so, the availability of these herbs are restricted in some countries, but available in other countries. Negative effects are occasionally posed by botanical supplements with contents of toxic constituents, such as liver toxins or carcinogens (chemicals with potential to cause cancer). However, as emphasized in rules, distribution of these supplements in markets will be stopped, once a potential problem is identified (Jaggi & Low, 2000).

2.4 Diabetes

Non-insulin-dependent diabetes mellitus has been rapidly rising in developing countries. This substance, which use is widespread in the developed Western world, is becoming an epidemic on the global level. Furthermore, type II diabetes has become two times more prevalent within 10 years, which is within 1995 to 2004 (Van Dieren *et al*., 2011). It has also been stated by The International Diabetes Federation that type 2 diabetes will be more prevalent, where it will increase from the currently estimated 285 million in 2010 to 438 million in 2030 (International Diabetes Federation, "Diabetes"). As of recently, there are approximately 113 million diabetes carriers who are living in urban areas. On the other hand, there are approximately 78 million diabetes carriers in rural areas. Nevertheless, it is predicted that in 2030 the increase ofdiabetes carriers in rural areas will be by less than a

quarter. However, the number of carriers in urban areas increase by two times (International Diabetes Federation, "Diabetes") (Yang *et al*., 2010).The number of diabetes patients was relatively the highest in India in 2002. However, China has recently overtaken India's place, with 15.5% of the population in this country being diabetic (Pradeepa *et al*., 2002; Yang *et al*., 2010). Furthermore, it has been indicated that obesity is the source of a number of non-communicable chronic diseases such as type 2 diabetes. It also results to cardiovascular diseases such as stroke, hypertension, and heart disease (Kumanyika *et al*., 2002). There has been a link between the rapid changes in dietary habits which start with the socioeconomic changes taken place in the urban areas of developing countries (e.g. India, China, and Latin America), and the prevalent type 2 diabetes in these regions (Pradeepa *et al*., 2002; Yang *et al*., 2010). Moreover, urbanization has been the factor of high-calorie diets where cereal and fibre intake has been scarce (Popkin, 2003). Not only that, physical activity has decreased since the invention of technology. Aside from resulting to a sedentary lifestyle, this has led to several complicated issues (Walker *et al*., 2010) reported that there is a possibility for type 2 diabetes to be reduced by 28% to 59% provided that diet and exercise are included in lifestyle. The risks of diabetes are not only in terms of the health risks, they also result to the economic burden which is shouldered by societies who are still fighting against infectious diseases (Yang *et al*., 2010). An approximate amount of $376 billion is spent for the current global health care expenditure, which aims for the prevention of diabetes and its complications. Nearly $0.5 trillion is expected to be achieved by 2030 (International Diabetes Federation, "Diabetes") (Walker *et al*., 2010).

2.4.1 Anti-diabetic compounds from natural product

Provided that drugs are always created from plants, the development of many of the currently available drugs are done directly or indirectly. Based on the report from ethnobotanical informationthere is a possibility that anti-diabetic potential is available within around 800 plants (Alarcon-Aguilara *et al*., 1998). Upon being assessed with the current experimental methods, anti-diabetic activity is displayed by these herbs (Mukherjee *et al*., 2006). A large group of plant-derived active principles present the activities which are based on the use suitable for the treatment of non-insulin-dependent diabetes mellitus. They are the indicator of numerous chemical compounds (NIDDM) (Marles & Farnsworth, 1995). Examples of the chemical compounds are inorganic ions, amino acids, terpenoids, glycopeptides, carbohydrates, steroids, guanidine, hypoglycans, peptidoglycans, polysaccharides, galactomannan gun, glycosides, and alkaloids. Besides, the presence of the frequently used hypoglycaemic drug metformin is a result of the traditional method where the use of Galega officinalis is involved. Therefore, although plants have the potential as the substance for anti-diabetic drugs (and other drugs, too), there is a lack of momentum obtained within the scientific community. Additionally, the significant decrease of blood glucose, glycosylated haemoglobin, and urea due to the ethanolic extract of *Ocimum tenuiflorum* L. This comes along with the increase of the protein within streptozotocin-induced diabetic rats, haemoglobin, and glycogen (Narendhirakannan *et al*. 2006). The rise in the levels of insulin and peptide, which is followed by the tolerance of glucose, are the outcomes of these extracts. There are several stimulatory effects caused by the constituents of *Ocimum tenuiflorum* L. leaf extracts (Hannan *et al*., 2006) on the physiological pathways of insulin secretion. This will lead to the reported antidiabetic action displayed by the insulin secretion.