



*IN VIVO* ANTIDIABETIC STUDIES OF WOGONIN  
ISOLATED FROM THE LEAVES OF *TETRACERA*  
*INDICA* MERR.

BY

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## ABSTRACT

The present thesis deals with the isolation, *in vivo* antidiabetic and toxicological evaluation of a flavonoid i.e. wogonin (5, 7-dihydroxy-8-methoxyflavone) isolated from the MeOH extract of *Tetracera indica* Merr. leaves (Dilleniaceae) (Mempelas paya in Malay) which is traditionally used in the management of diabetes in Malaysia. The thesis is therefore, presented in following three parts. Chemical investigation of the methanol extract (190 g) from the powdered leaves (1 kg) of *T. indica* resulted in the isolation of a desirable flavonoid i.e., wogonin (4.5 g). Isolation of the wogonin was carried out in three steps: multiple extractions with MeOH, fractionation using column chromatography and purification using recrystallization techniques with different polar solvents. Its structure was elucidated by spectroscopic analysis, mainly <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, MASS, IR and UV techniques as well as comparison of the spectral data was made with those reported in the literatures for the same compound. *In vivo* antidiabetic study of wogonin was carried out using Sprague-Dawley rats (diabetic as well as normal) at three different concentrations (1, 5 and 25 mg/kg b.w.) At 5 and 25 mg/kg b.w., wogonin was found to exhibit significant anti-hyperglycaemic activity ( $p < 0.05$ ) in alloxan induced diabetic rats. In normal rats, no hypoglycaemic activity was observed at all the concentrations, when compared with +ve and -ve controlled groups. The antidiabetic activity was found to be comparable with glibenclamide (GLBC) ( $p < 0.05$ ), a known oral hypoglycemic agent (5 mg/kg b.w.). *In vivo* toxicological study was performed using the same strain of rats at two different concentrations (5 and 25 mg/kg b.w.) for continuous 15 days. During the study period no abnormal activity and mortality were observed. Histopathology of kidney, liver and pancreas of both normal and diabetic treated rats demonstrated normal and improved condition when compared to diabetic control group. The present study was also carried out to evaluate the effects of wogonin on biochemical parameters such as- kidney functional parameters (serum urea and creatinine) and serum lipid profile (TC, TG, LDL-C and HDL-C) parameters. There were no significant changes seen in the kidney and lipid functional parameters tested. This study has showed the significant efficacy of wogonin against diabetes without showing any toxicity. It is concluded that wogonin isolated from the leaves of *T. indica* is a safe and effective antidiabetic compound and may provide a chemical lead for the synthesis of new derivatives which might prove to be potential novel therapeutic agents in the treatment of diabetes.

**Keywords:** *Tetracera indica* Merr. Dilleniaceae, isolation, wogonin, *in vivo*, antidiabetic activity, toxicological study, histopathology.

## خلاصة البحث

1 هذا البحث يهدف الي العزل في الجسم الحي كمضاد للسكري وتقييم السمية من الفلافونويد-7, 5 (dihydroxy-8-methoxyflavone) معزولة عن استخراج MeOH من *Tetracera* مؤشر على . Merr أوراق (Mempelas) (Dilleniaceae) بايا في الملايو (الذي يستخدم عادة في إدارة مرض السكري في ماليزيا. ولذلك، قدم أطروحة في أعقاب ثلاثة أجزاء. أسفر التحقيق الكيميائي لمستخلص الميثانول (190 غ) من الأوراق المسحوقة (1 كجم) من T. إندিকা في عزلة مرغوب فيه أي الفلافونويد، wogonin (4.5 غرام). وأجري عزلة wogonin في ثلاث خطوات: الاستخراج متعددة مع MeOH، تجزئة باستخدام اللوني العمود وتنقية باستخدام تقنيات التبلور مع المذيبات القطبية مختلفة. تم توضيح هيكلها عن طريق التحليل الطيفي، وذلك أساسا <sup>13</sup>C-NMR، H-NMR1، MASS، وتقنية IR والأشعة فوق البنفسجية وكذلك مقارنة البيانات الطيفية مع ما ورد في الابحاث السابقة لنفس المركب. في دراسة داخل الجسم الحي ل wogonin كمضاد لمرض السكر، أجريت على الفئران سبراغ داوولي (المريضة بالسكري والطبيعية) في ثلاثة تركيزات مختلفة (1 ملغ / كغ من وزن الجسم، 5 ملغم / كغم من وزن الجسم و 25 ملغ / كغ من وزن الجسم) في 5 و 25 ملغ / كغ من وزن الجسم، وقد وجد wogonin لعرض النشاط المضادة لل hyperglycaemic كبير في ألوكسان المعطي للجرذان المصابة بداء السكري المستحث. في الفئران العادية، لم يلاحظ أي نشاط خافض للسكر في جميع تركيزات، وذلك بالمقارنة مع مجموعات التحكم الموجبة والسالبة. تم العثور على النشاط المضاد لتكون قابلة للمقارنة مع غليسينيكلاميد (GLBC)، وكيل سكر الدم عن طريق الفم المعروفة (5 ملغ / كغ من وزن الجسم). في الجسم الحي تم إجراء الدراسة السمية باستخدام نفس سلالة من الفئران في اثنين من تركيزات مختلفة 5 و 25 mg / كغ من وزن الجسم مستمر لمدة خمسة عشر يوما. فترة الدراسة لم يلاحظ أي نشاط غير طبيعي أو وفيات. التشريح المرضي الكلى والكبد والبنكرياس كل من الفئران العادية والسكري تعامل أثبتت حالة طبيعية وتحسين بالمقارنة مع مجموعة السيطرة على السكري. وقد أجريت هذه الدراسة أيضا إلى تقييم آثار wogonin على القياسات البيوكيميائية مثل الكلى الفلك المعلمة وظيفية (اليوريا في الدم والكرياتينين) ومستوى الدهون في الدم TC، LDL-C و TG HDL-C لم تكن هناك تغييرات كبيرة ينظر في الكلى والمعلومات الوظيفية للدهون التي تم اختبارها. ووجدت الدراسة إلى فعالية كبيرة من wogonin ضد مرض السكري دون أن تظهر أي سمية. وخلص إلى أن wogonin معزولة من أوراق T. إندিকা هو مركب مضاد لمرض السكر آمن وفعال ويمكن أن توفر كقائد كيميائي لتصنيع مشتقات جديدة التي قد يثبت أن لها قدرة علاجية جديدة محتملة في علاج مرض السكري.

## APPROVAL PAGE

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## DECLARATION

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

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***IN VIVO* ANTIDIABETIC STUDIES OF WOGONIN ISOLATED FROM THE  
LEAVES OF *TETRACERA INDICA* MERR.**

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# TABLE OF CONTENTS

Abstract .....	ii
Abstract in Arabic.....	iii
Approval Page.....	iv
Declaration.....	v
Copyright Page.....	vi
Acknowledgements.....	vii
List of Tables .....	xi
List of Figures .....	xii
List of Equations .....	xvi
<b>CHAPTER ONE: INTRODUCTION .....</b>	<b>1</b>
1.1 Background of The Study.....	1
1.2 Medicinal Plants .....	3
1.3 <i>Tetracera Indica</i> Merr. ....	4
1.4 Wogonin (5, 7 –Dihydroxy-8-Methoxyflavone) .....	5
1.5 Purpose of The Study.....	6
1.6 General Objectives.....	6
1.7 Specific Objectives .....	7
1.8 Reaserch Hypothesis.....	7
1.9 Significance of The Study .....	7
<b>CHAPTER TWO: LITERATURE REVIEW.....</b>	<b>9</b>
2.1 Diabetes Mellitus: Definition and Description.....	9
2.2 Classification of Diabetes Mellitus.....	13
2.2.1 Type 1 Diabetes.....	14
2.2.1.1 Immune-Mediated Type 1 Diabetes .....	14
2.2.1.2 Idiopathic Type 1 Diabetes.....	15
2.2.2 Type 2 Diabetes Mellitus or Adult-Onset Diabetes Mellitus.....	15
2.2.3 Other Earmarked Categories of Diabetes.....	16
2.2.3.1 Genetic Defects of The B-Cell .....	16
2.2.3.2 Genetic Defects in Insulin Function .....	17
2.2.3.3 Diseases of The Exocrine Pancreas .....	17
2.2.3.4 Endocrinopathies .....	19
2.2.3.5 Drug or Chemical-Induced Diabetes .....	20
2.2.3.6 Viral Infection .....	21
2.3 Oral Anti-Diabetic Medications .....	21
2.3.1 Metformin (Biguanides).....	22
2.3.2 Sulfonylureas .....	22
2.3.3 Thiazolidinedions.....	23
2.3.4 A-Glucosidase Inhibitors .....	24
2.3.5 A-Amylase Inhibitors.....	25
2.3.6 Dipeptidyl Peptidase-IV Inhibitors .....	25
2.4 Treatment of Diabetes With Insulin Therapies.....	26
2.5 Adverse Effects of Antidiabetic Drugs.....	27
2.6 Role of Herbal Medicine .....	28



2.7 Antidiabetic Compounds From Plants.....	29
2.8 Role of Flavonoids in Diabetes .....	32
2.9 <i>Tetracera</i> Genus .....	35
2.9.1 <i>Tetracera Indica</i> (Houtt. Ex Christm. & Panz) Merr.....	36
2.9.2 Scientific Classification of <i>T. Indica</i> Merr.....	37
2.9.3 Traditional Uses of <i>T. Indica</i> .....	37
2.9.4 Experimental Evidences of <i>T. Indica</i> .....	38
2.9.5 Phytochemical Investigation of <i>T. Indica</i> .....	39
2.10 Wogonin (5, 7–Dihydroxy-8-Methoxyflavone) .....	39
<b>CHAPTER THREE: METHODOLOGY.....</b>	<b>41</b>
3.1 Experimental Material .....	41
3.1.1 Collection and Preparation of Plant Material.....	41
3.1.2 Chemicals .....	42
3.2 Experimental Methods.....	43
3.2.1 Preparation of Methanol Extract (MeOH) of The Leaves of <i>T. Indica</i> .....	43
3.2.2 Isolation And Purification of Wogonin From The Methanol Extract of Leaves of <i>T. Indica</i> .....	44
3.3 Experimental Animals .....	49
3.3.1 Biochemical Parameters.....	50
3.3.2 Animal Toxicity Study of Wogonin By Using OECD, 425 Guideline .....	50
3.3.3 Experimental Design For Diabetic Rats.....	51
3.3.4 Induction of Experimental Diabetes.....	53
3.3.5 Oral Glucose Tolerance Test (OGTT) .....	54
3.4 Histopathological Preparation of Samples.....	55
3.5 Laboratory Analysis of Biochemical Parameters .....	58
3.5.1 Renal Functional Test (Serum Urea and Creatinine) .....	58
3.5.1.1 Serum Urea Concentration (mmol/L).....	58
3.5.1.2 Serum Creatinine Concentration ( $\mu\text{mol/L}$ ).....	58
3.5.2 Estimation of Lipid Parameters.....	59
3.5.2.1 Total Cholesterol (TC) .....	59
3.5.2.2 Triglycerides (TG).....	59
3.5.2.3 High Density Lipoprotein Cholesterol (HDL-C) .....	59
3.5.2.4 Low Density Lipoproteins Cholesterol (LDL-C).....	60
3.6 Statistical Analysis.....	60
<b>CHAPTER FOUR: RESULTS .....</b>	<b>63</b>
4.1 Preparation of Methanol Extract.....	63
4.2 Isolation and Purification of Wogonin From Methanol Extract of The Leaves of <i>T. Indica</i> .....	63
4.2.1 Wogonin (MA-1) .....	63
4.2.2 Ultraviolet Radiation (UV) .....	63
4.2.3 Infrared Radiation (IR).....	68
4.2.4 Mass Spectrometry.....	69
4.3 OGTT of Wogonin in Alloxan Induced Diabetic Groups .....	73
4.4 Estimation of Safety Profile of Wogonin .....	75
4.5 <i>In Vivo</i> Antidiabetic Activity of Wogonin in Experimental Rats.....	82

4.5.1 Histopathological Evaluation of Rat Nephritic, Hepatic and Pancreatic Tissues in Experimental Animals .....	84
4.6 Effect of Wogonin on Body Weight Changes in Diabetic Control and Diabetic Treated Rats .....	94
4.7 Effect of Wogonin on Kidney Function Parameters in Normal and Alloxan Induced Diabetic Rats.....	96
4.8 Effect of Wogonin on Total Serum Lipid Profile Parameters in Normal and Alloxan Induced Diabetic Rats.....	99
<b>CHAPTER FIVE: DISCUSSION.....</b>	<b>103</b>
5.1 Isolation and Structure Elucidation of Wogonin Isolated From The Leaves of <i>Tetracera Indica</i> Merr. ....	103
5.2 Structure Elucidation of Wogonin (MA-1): .....	103
5.3 Animal Toxicity Study of Wogonin Using OECD, 425 Guideline .....	107
5.4 Histopathological Analysis .....	108
5.5 <i>In Vivo</i> Antidiabetic Activity of Wogonin Isolated From The Leaves of <i>T. Indica</i> .....	109
5.6 Effect of Wogonin on Body Weight Changes in All Groups .....	112
5.7 Effect of Wogonin on Kidney Functional Parameters.....	112
5.8 Effect of Wogonin on Lipid Profile Parameters .....	113
<b>CHAPTER SIX: CONCLUSION.....</b>	<b>114</b>
<b>REFERENCES.....</b>	<b>115</b>
<b>APPENDIX A .....</b>	<b>130</b>
<b>APPENDIX B .....</b>	<b>134</b>
<b>APPENDIX C .....</b>	<b>135</b>
<b>APPENDIX D .....</b>	<b>137</b>

## LIST OF TABLES

<u>Table No.</u>		<u>Page No.</u>
2.1	Glycaemia syndrome-etiological types and stages	12
2.2	Estimated degree of diabetes mellitus in various categories of pancreatic disease	18
3.1	Animal groups	53
4.1	OGTT values of diabetic control, diabetic treated with wogonin (1,5 and 25 mg) and GLBC (5 mg) at different time intervals	74
4.2	Safety profile estimation data on normal rats after treatment with 5 and 25 mg/kg b.w. of wogonin (15 days)	76
4.3	CNS toxicity and abnormal physical activities estimation data on normal rats after treatment with 5 and 25 mg/kg b.w. of wogonin (15 days)	77
4.4	Effect of different doses of wogonin (5, 7-dihydroxy-8-methoxyflavone) on blood glucose levels (mmol/L) in normal, normal treated, diabetic control and diabetic treated rats in every alternate days (two weeks)	83
4.5	Effect of wogonin on body weight (g) in every alternate days (two weeks) in normal control, normal treated, diabetic control and diabetic treated rats	95
4.6	Effect of wogonin on kidney functional parameters in normal, normal treated, diabetic control and diabetic treated with wogonin	97
4.7	Plasma total cholesterol (TC), triglycerides (TG), high-density cholesterol (HDL-C) and low-density cholesterol (LDL-C) concentrations in control and experimental groups of rat	100
5.1	<sup>1</sup> H and <sup>13</sup> C-NMR (Acetone, D6, 150 MHz) of MA-1 in comparison with (Harrison et al., 1994)	106

## LIST OF FIGURES

<u>Figure No.</u>		<u>Page No.</u>
1.1	Wogonin (5, 7-dihydroxy-8-methoxyflavone)	6
2.1	Schematic diagram of an pancreatic Islets of Langerhans	20
2.2	Metformin	22
2.3	Pioglitazone	24
2.4	Rosiglitazone	24
2.5	Acarbose	25
2.6	Voglibose	25
2.7	Sitagliptin	26
2.8	Berberine	30
2.9	Gallic acid	30
2.10	Quercetin	31
2.11	Catechin	32
2.12	Basic structure of flavonoid	32
2.13	<i>Tetracera indica</i> Merr. (leaves and flower)	36
2.14	Wogonin (5, 7-dihydroxy-8-methoxyflavone)	39
3.1	Dried leaves of <i>T. indica</i>	41
3.2	Pulverization of dried leaves through Fritsch Universal Cutting Mill-PULVERISETTE 19-Germany	42
3.3	Concentration of extract through Buchi rotary evaporator	44
3.4	Combined fractions were mixed and purified to get the yellow color crystallized compound	46
3.5	Isolated pure form of compound MA-1 (wogonin)	46
3.6	Schematic diagram of isolation process of pure wogonin	48

3.7	Male albino rat of Sprague Dawley strain (SD)	49
3.8	Plastic blocks containing sample specimens	56
3.9	Slides containing tissues kept onto hotplate at room temperature	56
3.10	After staining, the slides of kidney, pancreas and liver	57
3.11	Schematic diagram of <i>in vivo</i> studies of wogonin	62
4.1	UV spectrum of wogonin in Methanol (MeOH)	64
4.2	UV spectrum of wogonin in NaOMe/MeOH	65
4.3	UV spectrum of wogonin in NaOMe/MeOH (after 10 minutes)	65
4.4	UV spectrum of wogonin in NaOH/MeOH	66
4.5	UV spectrum of wogonin in NaOAc/MeOH	66
4.6	UV spectrum of wogonin in NaOAc/H <sub>3</sub> BO <sub>3</sub> /MeOH	67
4.7	UV spectrum of wogonin in AlCl <sub>3</sub> /MeOH	67
4.8	UV spectrum of wogonin in AlCl <sub>3</sub> /HCl	68
4.9	IR spectrum of wogonin	69
4.10	GC-MS of MA-1	70
4.11	<sup>1</sup> H-NMR spectrum of wogonin	71
4.12	<sup>13</sup> C-NMR (1H-decoupled) spectrum of wogonin	72
4.13	<sup>13</sup> C-NMR (apt) spectrum of wogonin	73
4.14	Changes have been observed and comparison has made between normal rat's kidney tissue before (a) and after (b and c) wogonin (5 and 25 mg) treatment	79
4.15	Changes have been observed and comparison has made between normal rat's liver cell before (a) and after (b and c) wogonin (5 and 25 mg) treatment.	80
4.16	Changes have been observed and comparison has made between normal rat's pancreatic tissue before (a) and after (b and c) wogonin (5 and 25 mg) treatment.	82
4.17	Percentage fall of blood glucose level of different doses of wogonin in normal control, normal treated, diabetic treated and diabetic control group of rats	84

4.18	Kidney tissue of alloxan-induced diabetic rats without any treatment (H & E×40 magnification)	86
4.19	Kidney section of alloxan-induced diabetic rats after treatment with 5 mg/kg b.w. of glibenclamide (GLBC) showing no abnormal changes (H & E×40 magnification)	86
4.20	Kidney section of alloxan-induced diabetic rats after treatment with 1 mg/kg b.w. (low dose) of wogonin demonstrated normal glomerular structure (H & E×40 magnification)	87
4.21	Kidney section of alloxan-induced diabetic rats after treatment with 5 mg/kg b.w. (medium dose) of wogonin exhibited improved conditions (H & E×40 magnification)	87
4.22	Kidney section of alloxan-induced diabetic rats after treatment with 25 mg/kg b.w. (higher dose) of wogonin (H & E × 40 magnification)	88
4.23	Liver tissue section of diabetic rats without treatment demonstrated very little changes in liver cells (H & E×40 magnification)	88
4.24	Liver tissue section of diabetic rats after treatment with 5 mg/kg b.w. of GLBC and demonstrated normal liver cells (H & E×40 magnification)	89
4.25	Liver tissue section of diabetic rats after treatment with 1 mg/kg b.w. (low dose) of wogonin showed normal liver cell structure (H & E×40 magnification)	89
4.26	Liver tissue section of diabetic rats after treatment with 5 mg/kg b.w. (medium dose) of wogonin exhibited normal liver cell structure without any adverse signs (H & E×40 magnification)	90
4.27	Liver tissue section of diabetic rats after treatment with 25 mg/kg b.w. (higher dose) of wogonin demonstrated normal cytoplasm structure and observed absence of any significant adverse effects (H & E×40 magnification)	90
4.28	Pancreatic tissue section of diabetic rats without treatment demonstrated mild damages in pancreatic beta cells after two weeks (H & E×40 magnification)	91
4.29	Pancreatic tissue section of diabetic rats after treatment with 5 mg/kg b.w. of GLBC demonstrated normal pancreatic beta cells after two weeks (H & E×40 magnification)	92
4.30	Histopathological examination of pancreatic tissue of diabetic treated rats with low dose (1 mg/kg b.w.) of wogonin showed absence of pancreas damage (H & E×40 magnification)	92

4.31	Pancreatic tissue section of diabetic treated rats after treatment with medium dose of wogonin at 5 mg/kg b.w. showed no damages on islet of Langerhans in pancreatic $\beta$ -cells (H & E $\times$ 40 magnification)	93
4.32	Histopathological appearances from the pancreatic tissue of diabetic rats after treatment with higher dose of 25 mg/kg b.w. of wogonin (H & E $\times$ 40 magnification)	93
4.33	Effect of different doses of wogonin on body weight (g) of all groups throughout the entire study period	96
4.34	The comparison in values of serum urea concentration (mmol/L) among all groups of rat	98
4.35	The comparison in values of serum creatinine concentration ( $\mu$ mol/L) among all groups of rat	98
4.36	The comparison of plasma cholesterol (TC) concentration (mmol/L) among all groups of rat	100
4.37	The comparison of triglycerides (TG) concentration (mmol/L) among all groups of rat	101
4.38	The comparison of HDL-C cholesterol concentration among all groups of rat	102
4.39	The comparison of LDL-C cholesterol concentration among all groups of rat	102
5.1	Wogonin (5, 7-dihydroxy-8-methoxyflavone) (MA-1)	105

## LIST OF EQUATIONS

<u>Equation No.</u>	<u>Page No.</u>
3.1	52



# CHAPTER ONE

## INTRODUCTION

### 1.1 BACKGROUND OF THE STUDY

Diabetes mellitus (DM) is a group of metabolic and endocrine disorder characterized by hypoglycemia resulting from defects in insulin secretion, insulin action or both (WHO, 2012; Teixeira et al., 2000). This is a global health problem and according to WHO (World Health Organization) investigation, number of diabetic patients is expected to rise up from 171 million in year 2000 to 366 million or more by the year 2030 and about 90% to 95% of all cases of diabetes were type 2 diabetes mellitus. DM has been declared by WHO as epidemic and mostly affecting regions with diabetes are Asia and Africa, where DM rates are expected to increase by 2-3 folds than the present rates by the year 2030 (ADA, 1997; Wild et al., 2004; Shaw et al., 2010). This disease requires expensive treatment and the costs associated with the treatment of diabetes and its complications are estimated to exceed \$200 billion a year worldwide. Diabetes is mainly developed by the inadequate function of pancreas usually when the pancreas fails to produce enough insulin or the insulin which is produced by the pancreas is not well utilized in body (WHO, 2013). DM is classified as Type 1 and Type 2 DM. Type 1 DM or (insulin dependent diabetes mellitus) is commonly seen in juveniles, usually developed by failure to produce insulin due to autoimmune destruction of  $\beta$ -cells of the pancreas while type 2 DM or (non-insulin dependent diabetes mellitus) is usually adult onset and is associated with insufficient production of insulin and loss of responsiveness by cells to insulin (Hawk and Bernard, 1954; Yallow et al., 1960). Currently, diabetes has become the third 'killer' disease along with cancer, cardiovascular and cerebrovascular diseases because of its high

prevalence, morbidity and mortality and this life threatening disease itself alone is accompanied with several other diseases infecting healthy individuals. At present, the available therapeutic choices for non-insulin-dependent diabetes mellitus (NIDDM) are such as dietary modification, oral hypoglycaemic, and insulin and these are not only expensive to manage but also have some limitations of their own. Many of these anti-diabetic agents have a number of serious adverse effects on health, thus management of diabetes without any side effects is still big challenge to the medical community. The use of the drugs is restricted by their pharmacokinetic properties, secondary failure rates and accompanying side effects (Vishwakarma et al., 2010).

In Malaysia, first survey on the epidemiology of diabetes conducted by National Health and Morbidity Survey (NHMS I) in 1986 revealed the prevalence of diabetes about 6.3%, which escalated to 8.3% by the year 1996 reported in the Second National Health and Morbidity Survey report (NHMS II).

In 2006, the third survey on the epidemiology of diabetes third National Health and Morbidity Survey (NHMS III) further demonstrated the high prevalence rate of diabetes and impaired fasting glycaemia (IFG) among Malaysian populations. The prevalence rate of diabetes was found to be 11.6% among the people above 18 years old and 14.9% for the age more than 30 (NHMS, 1996).

Anti-diabetic drugs treat diabetes by lowering glucose levels in the blood. With the exceptions of insulin, exenatide, and pramlintide all anti-diabetic drugs are administered orally and are thus also called oral hypoglycaemic agents or oral antihyperglycemic agents. Selection and classification of anti-diabetic drugs depends on the nature of the diabetes, age and situation of the person, as well as other factors. Treatment for diabetes includes- (1) agents which accelerate the amount of insulin secreted by the pancreas, (2) agents which increase the sensitivity of target organs to

insulin and (3) agents which reduces the rate at which glucose is absorbed from the gastrointestinal tract (Rendell, 2004).

## **1.2 MEDICINAL PLANTS**

The search for a new class of compounds is very important to overcome diabetes and its related infirmities. Therefore, there should always be a continuous search for alternative and safe drugs. Current estimates from different countries in Europe and the US have shown that diabetes and its related complications account for 8-16% of the total health costs for society and this will expand dramatically unless major efforts are made to forestall the ongoing epidemic (Brahmachari, 2011). Medicinal plants are considered the best source to obtain a variety of drugs according to the World Health Organization (Santos et al., 1995). To treat hypoglycemic and hyperglycemic 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 hypoglycemic and anti-hyperglycemic effects as well (Grover et al., 2002). In many medicinal plant species, the active principles with desired properties have been isolated to cure ailments such as type-1 and type-2 DM (Fabricant and Farnsworth, 2001).

Medicinal plants are the most precious gift from the nature to the mankind. These plants play an important role for various diseases and are found to be grown in different climatic conditions. Many rural places rely on medicinal plants for their drug origin as well as subsistence. The medicinally active substances existent in all these medicinal plants have already been claimed for their therapeutic efficaciousness (Havsteen, 2002; Middleton et al., 2000). More than 1000 plant species are being used

globally for the treatment of type 2 DM (Trojan-Rodrigues et al., 2011). From medicinal plants so many vital bioactive constituents like dietary fibers, flavonoids, alkaloids, terpenoids, saponins, amino acids etc. have been isolated and also been proven for their hypoglycemic nature either by rising up glucose utilization or by releasing insulin from pancreatic  $\beta$ -cells and inhibiting glucose absorption in gut (Li et al., 2004; Modak et al., 2007).

On this earth around 1 to 10% of plants are utilized by mankind out of estimated 250,000 to 500,000 plant species (Borris, 1996). The chemical diversity of natural product fulfilling to the variety found in synthetic libraries. Due to long evolutionary selection process, natural products have greater ring system variety and are more complicated. Therefore, strategies to take advantage of natural origins and to develop methodologies for natural product preparation such as libraries are probable by using combinational biosynthesis and related techniques (Lewis and Lewis, 1995).

### **1.3 TETRACERA INDICA MERR.**

*Tetracera indica* Merr. (Synonyms: Houtt. Ex Christm. and Panz) (Family: DILLENACEAE) is a large, woody, rain forest climber of Malaysia which is commonly known as Empelas, Hempelas, Mempelas, sand paper plant, Mempelas minyak or Mempelas paya in Malay. Leaves are simple, with 6-10 x 3-5 cm, elliptical to oblong. Flowers are usually 2.5-3 cm wide with reddish white petals and above stamens are red and white in below. The flowers also have a nice fragrance. Fruits are capsules, globular, about 1 cm wide and 2-6 mm bark, 2 or more seeds, each with 1 cm long aril that is bright in red, it tastes kind of sour and look like berries. It is widely distributed from Myanmar southwards to Thailand, Indochina, Peninsular Malaysia, Sumatra, Bangka, Java and Madura (Tawan, 2001; Chong et al., 2009).

In folk Malaysian medicines, the leaves and roots of *T. indica* are used to treat various ailments including diabetes. The leaves and roots are pounded and applied to the itchy allergic skin to alleviate pain. The leaves are also used to lessen high blood pressure and high fever (Ong and Nordiana, 1999). Moreover, Malaysians also use it to treat headache by using its pulverized shoot wrapped in banana leaves heated and then applied (Latiff and Zakri, 1996). Furthermore, *T. indica* is also one of the active ingredients in a local herbal medicine called ‘plantisol’ which is widely prescribed by the local herbalists in Malaysia to treat diabetes. *Barringtonia racemosa*, *Pithecellobiumjiringa*, *Tinospora crispa* and *Andrographis paniculata* are the other active ingredients of Plantisol medicine (Mayat, 2009). Based on its traditional claims, it is one of the good candidates to tackle aforementioned problems associated with chronic diabetes. Phytochemical screening of *T. indica* leaves has revealed the presence of four terpenoids viz.,  $\beta$ -sitosterol, lupeol, betuline, betulinic acid (Dan and Dan, 1980) and a flavonoid named as wogonin (5, 7-dihydroxy-8-methoxyflavone) (Harrison et al., 1994; Tawan, 2001).

#### **1.4 WOGONIN (5, 7 –DIHYDROXY-8-METHOXYFLAVONE)**

Wogonin (5, 7-dihydroxy-8-methoxyflavone) is one of the major compounds found in the leaves of *T. indica*.

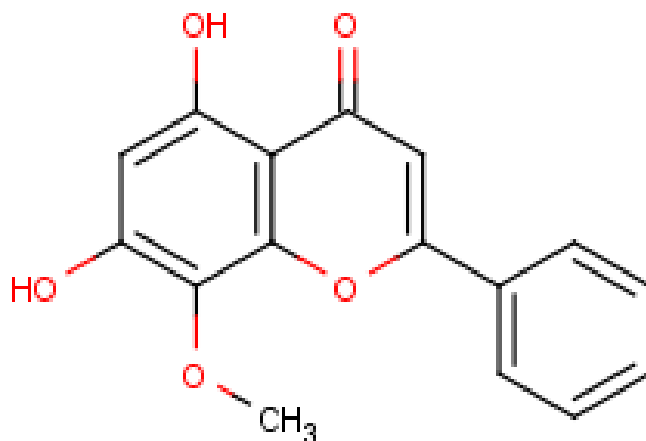


Figure 1.1 Wogonin (5, 7-dihydroxy-8-methoxyflavone)

### 1.5 PURPOSE OF THE STUDY

Being a major constituent of the leaves of *T. indica*, wogonin could possess or exert antidiabetic potential. Hence, the purpose of this study was to investigate the potential anti-diabetic action of pure wogonin that exists as one of the major compounds in the leaves of *T. indica* which are traditionally used to treat diabetes in several parts of Malaysia. Moreover, different doses of this compound will be given to *in vivo* animal model to see whether any side effect could occur or not as well as to check at what minimum doses this compound could exert its blood glucose lowering effects or anti-diabetic effect.

### 1.6 GENERAL OBJECTIVES

To isolate pure wogonin (5, 7-dihydroxy-8-methoxyflavone) from the leaves of *T. indica* using different analytical techniques in good yield, and to investigate *in vivo* antidiabetic potential and toxicological nature of this compound.

## **1.7 SPECIFIC OBJECTIVES**

The study aimed to achieve the following specific objectives:

1. To isolate wogonin in pure and higher yield from the methanol extract of *T. indica*'s leaves through analytical techniques.
2. To characterize the structure of wogonin through different spectroscopic techniques (UV, IR, MS and <sup>1</sup>H-NMR, <sup>13</sup>C-NMR).
3. To investigate *in vivo* effect of wogonin on postprandial blood glucose levels after oral glucose loading (OGTT) in alloxan-induced diabetic rats.
4. To investigate *in vivo* effects of wogonin on fasting blood glucose, body weight and lipid parameters in normal and alloxan-induced diabetic rats.
5. To evaluate toxicity of wogonin through histopathology in diabetes affected organs (kidney, liver and pancreas) by using Up and Down method followed by OECD 425 guidelines.

## **1.8 REASERCH HYPOTHESIS**

Wogonin (5, 7-dihydroxy-8-methoxyflavone) as an active principle is responsible for the antidiabetic effect of *T. indica*'s leaves.

## **1.9 SIGNIFICANCE OF THE STUDY**

Wogonin (5, 7-dihydroxy-8-methoxyflavone) as one of the major compounds in the leaves of *T. indica* might be responsible for the antidiabetic effect of the leaves of *T. indica*. Hence, wogonin as a principle agent might exert anti-diabetic effect in rats and consequently could be a promising anti-diabetic agent for future clinical studies associated with diabetes. Histopathology studies can provide its safe or toxic nature in affected organs because of diabetes. Determination of its safety profile could help us

to understand whether it could be efficaciously used in future in the management of diabetes.