# PHYTOCHEMICAL AND ANTIDIABETIC INVESTIGATIONS OF "*TETRACERA SCANDENS*" LINN.

BY

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A thesis submitted in fulfilment of the requirement for the degree of Masters of Science in Pharmaceutical Chemistry

Kulliyyah of Pharmacy

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## ABSTRACT

Different parts (leaves, stems and roots) of *T. scandens* L. are used in folk remedies by various indigenous peoples in different countries for the treatment of rheumatism, lowering hypertension, lowering blood pressure, inflammatory diseases, hepatitis, internal pains, urinary disorders, dysentery, child birth, sore throat, gout and diabetes related infirmities. The present work investigates the anti-diabetic activity of the polar and non-polar extracts of *T. scandens* L. leaves *in vivo* in alloxan induced diabetic and normal rats. This study was conducted *in vivo* and comparison was made with standard antidiabeteic drug, glibenclamide (GLBC). Glucose levels in male albino rats (Wister strain) with hyperglycemia induced by alloxan (160 mg/kg b.w.) were determined after the oral administration of the aqueous (AQ) methanolic (MEOH), butanol (BuOH), ethylacetate (EtOAc) and dichloromethane (DCM) extracts. Four doses of AQ and MEOH extract (250, 500, 1000 and 2000 mg/kg b.w.) and a fixed dose of 500mg/kg b.w of BuOH, EtOAc and DCM extracts were evaluated. All extracts exhibited significant anti-hyperglycemic activity in alloxan induced diabetic rats, except DCM, however in normal rats no hypoglycemic activity was observed among all the extracts, when compared with both +ve & –ve controlled . The antidiabetic activity was found to be comparable to that of the effect produced by GLBC (0.25 mg/kg b.w.). The $LD_{50}$ of both AQ and MEOH extracts was found to be more than 5000 mg/kg body weight and no lethal toxicity was observed within this range. This study provides scientific evidence about the leaves of *T. scandens* L. which possess antidiabetic agents and justifies its utility by the local herbalists to treat diabetes in Malaysia. Phytochemical investigation and chromatographic fractionation of MEOH extract of the leaves of *T. scandens* L. led to the isolation and structure elucidation of two new and three known flavonoids, and two known terpenoids. The occurrence of kaempferol, quercetin, isoscutellarein, 5,7,8,3',5,-pentahydroxyflavone, 2, 3, 5, 6, 4'-pentahydroxy stilbene-(4→O→4''')-kaempferol-3''-*O*-*β*-D-glucopyranoside, stigmasterol and betullinic acid in the leaves of *T. scandens* L. is being reported for the first time.



**Keywords:** *Tetracera scandens* Linn., Dilleniaceae, Antidiabetic activity, Phytoconstituents

# خلاصة البحث


يُستخدم العديد من أجزاء ال *T. scandens* L. (الأوراق، السوق والجذور) في الأدوية العشبية المستخدمة من قبل السكان الأصليين في العديد من البلدان لمعالجة الرثية، خفض ارتفاع الضغط، خفض ضغط الدم، الأمراض الالتهابية، التهاب الكبد، الآلام الداخلية، الاضطرابات البولية، الزحار، عند الولادة، التهاب الحلق، النقرس والعيوب المرتبطة بالداء السكري. يتحرى العمل الحالي الفعالية المضادة لارتفاع السكر للخلاصات القطبية لأوراق *T. scandens* في الجسم الحي في الجرذان المصابة بداء السكري المحرَّض بالألوكسان وفي الجرذان السليمة. تمت هذه الدراسة في الجسم الحي وأُجريت المقارنة مع دواء مرجعي مضاد للسكري هو glibenclamide (GLBC). تم قياس مستويات الغوكوز عند ذكور الجرذان البيضاء (Wister strain) المصابة بارتفاع السكر المحرض بالألوكسان (160 mg/kg b.w.) بعد الإعطاء الفموي للخلاصات المائية (AQ)، الميتانولية (MEOH)، البوتانولية (BuOH)، خلاة الإيتيل أسيتات (EtOAC) والديكلوروميتان (DCM). تم تقييم أربع جرعات من الخلاصات المائية والميتانولية ( 250, 500, 1000 and 2000 mg/kg b.w.) وجرعة ثابتة من الخلاصة البوتانولية ( 500mg/kg b.w). كل الخلاصات أظهرت فعالية مهمة مضادة لارتفاع السكر في الجرذان المصابة بداء السكري المحرض بالألوكسان، باستثناء خلاصة الديكلوروميتان، لكن لم يلاحظ أي فعالية خافضة للسكر في الجرذان السليمة في كل الخلاصات، وذلك مقارنة بالشاهد +ve & –ve. تبين ايضاً أن الفعالية المضادة للسكري يمكن مقارنتها بتلك المحدثة بالGLBC (0.25 mg/kg b.w.). الـ $LD_{50}$ للخلاصتين المائية والميتانولية وُجدت أكثر من 5000 mg/kg من وزن الجسم ولم يلاحظ أية سُمّية قاتلة ضمن هذا المجال. إن هذه الدراسة توفر بوضوح دليلاً حول أوراق ؟؟؟ التي تملك عوامل مضادة للسكري وتفسر استخدامها من قبل المعالجين بالأعشاب المحليين لمعالجة الداء السكري في ماليزيا. التحريات الفيتوكيميائية والتجزئة للخلاصة الميتانولية لأوراق ؟؟؟ قادت إلى عزل وتحديد البنية لثلاثة فلافونوئيدات جديدة واثنين معروفين، وكذلك مركبين معروفين من التربينوئيدات. هذه هي المرة الأولى التي يُنشر فيها وجود الKaempferol ، Quercetin، isoscutellarein، 2, 5, 7, 8, 3', 5'-,pentahydroxyflavone 3, 5, 6, 4'-pentahydroxy stilbene-(4→O→4''')-kaempferol-3''-*O*-*β*-D-glucopyranoside (Stigmasterol) و (Betullinic acid) في أوراق *T. scandens* L.



الكلمات المفتاحية : *Tetracera scandens* Linn., Dilleniaceae, الفعالية المضادة للسكري، المكونات النباتية

# APPROVAL PAGE

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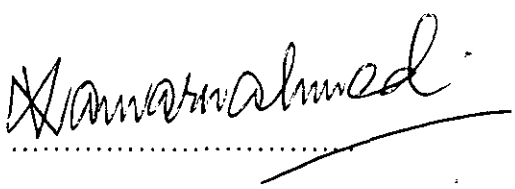

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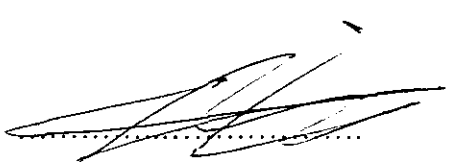

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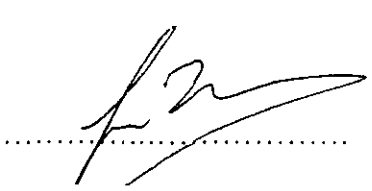

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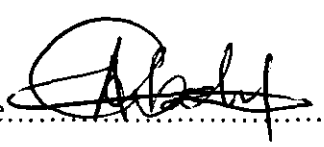
Signature  Date 20/04/11

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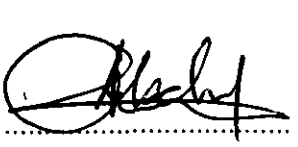

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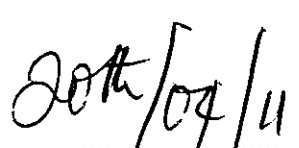

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*To my beloved and respected family of Usmaniyya, thank you all for your kind support love and care. Special thank to you dear great mum, your selfless spiritual imparted moral support have always been with me helping me in achieving my goals. Tons of thanks to my beloved dad, you've sacrificed both personally and professionally for me to chase down my dreams. I have to express my feelings of appreciations to you my dear respected grandma, even though my tongue is unable to pronounce enough words to praise for your wonderful spiritual advices, most especially if I remember your love towards me, advice, Islamic motivations, and all such beautiful words that always come out of your mouth that stir the love of Allah Almighty and His Most beloved Messenger Muhammad (SAW) in the depth of my heart. I hope you know how much it has meant to me and how important you are to me, blessings of Allah (SWA) be always with your souls of the lovers of Prophet Muhammad SAW.*

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Adulrashid Umar.

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| | |
|---|---|
| Anova | Analysis of Variance |
| $AlCl_3$ | Aluminum chloride |
| BuoH | Butanol |
| DCM | Dichloromethane |
| DM | Diabetes Mellitus |
| $Mgcl_2$ | Magnesium Chloride |
| MS | Mass spectrometry |
| ppm | parts per million |
| SAR | Structure Activity Relationship |
| TMS | Trimethylsilane |
| TLC | Thin Layer Chromatography |
| FA | Formic acid |
| nm | Nanometer |
| UV | Ultraviolet |
| $R_f$ | Retention factor |
| MeoH | Methanol |
| EtoH | Ethanol |
| EtoAc | Ethyl acetate |
| $^1H$ | Proton |
| $^{13}C$ | Carbon 13 |
| NMR | Nuclear Magnetic Resonance |
| IR | Infrared |
| KBr | Potassium bromide |
| NaoMe | Sodium methoxide |
| NaoAc | Sodium actetate |
| Me | Methyl group |
| Hz | Hertz |
| MHz | Mega hertz |
| $H_3BO_3$ | Boric acid |
| HCl | Hydrochloric acid |
| $H_2SO_4$ | Sulphuric acid |
| NaoH | Sodium hydroxide |
| Nacl | Sodium chloride |
| $Na_2CO_3$ | Sodium carbonate |
| $Na_2SO_4$ | Sodium sulphate |
| ml | milliliter |
| mg/kg | milligram/kilogram |
| mg/ml | milligram/kilogram |
| 1M | One molar |
| mmol | millimole |
| o | *ortho* |
| m | *meta* |
| p | *para* |
| $V/H_2SO_4$ | Vannilin/ Sulphuric acid |
| $FeCl_3$ | Ferric Chloride |

| | |
|---|---|
| QE | Quercetin equivalent |
| GAE | Gallic acid equivalent |
| Su | Sulfonylurea |
| BG | Biguanide |
| α- GDI | A-Glucosidase inhibitors |
| ARI | Aldose reductase inhibitors |
| TDZ | Thiazolidinediones |
| CMBA | Carbamoylmethyl benzoic acid |
| IGF | Insulin-like growth factor |
| IDDM | Insulin dependent diabetes mellitus |
| NIDDM | Non insulin dependent diabetes mellitus |
| WHO | World Health Organization |
| $LD_{50}$ | Medium lethal dose |
| SPSS | Statistical package for social sciences |
| OGTT | Oral glucose tolerance test |
| GLBC | Glibenclamide |
| FCR | Folin-ciocalceu reagent |

# CHAPTER ONE

# INTRODUCTION

## 1.1 BACKGROUND AND JUSTIFICATION

Diabetes mellitus is a metabolic disease which is characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The vast majority of cases of diabetes fall into two broad etiopathogenetic categories. In one category, type-1 diabetes, in which the cause is an absolute deficiency of insulin secretion. While the other is much more prevalent category, which is type-2 diabetes, where by the cause is a combination of resistance to insulin action as well as inadequate compensatory insulin-secretory response (Li et al., 2004).

Type-1 insulin-dependent diabetes mellitus (IDDM), in which the body does not produce any insulin, most often occurs in children and young adults. It accounts for 5-10% of diabetes (Jeffery et al., 2003).

Type-2, non insulin-dependent diabetes mellitus (NIDDM), in which the body does not produce enough, or properly use, insulin, is the most common form of the disease, accounting for 90-95% of the dreadening disease of diabetes. Type-2 diabetes is nearing epidemic proportions, due to an increased number of elderly people, and a greater prevalence of obesity and sedentary lifestyles. As a very common chronic disease, diabetes is becoming the third "killer" of the health of mankind along with cancer, cardiovascular and cerebrovascular diseases because of its high prevalence, morbidity and mortality (Li et al., 2004).

The cause of diabetes is a mystery, although both genetic and environmental factors such as obesity and lack of exercise appear to play a role. Ethnic and racial

differences have been found in heterogeneous populations within the same area. As a rule, incidence is highest in Scandinavian countries, intermediate in the US, Spain and lowest in Asian and most Latin American countries. With long course and serious complications often resulting in high death-rate, the treatment of diabetes spent vast amounts of resources including medicines, diets, physical training and so on in all countries (Li et al., 2004).

According to the WHO estimate, the number of diabetics will increase to 300 million by year 2025 (Jeffery et al.. 2003). Total health care spending on the disease worldwide is estimated to be US$ 213 billion and US$ 396 billion by the year 2025. It was predicted that 75% to be from developing countries because of rapid cultural and social changes and also increasing urbanisation, with this scenario, diabetes will burden the health care system, which is already strained with other chronic diseases such as coronary heart disease, asthma, hypertension, kidney failure etc. Adequate blood glucose control is vital in diabetes management to prevent complications. Yet, despite the various interventions, diabetes control remains a global problem to health care professionals (HCPs). More people suffer from diabetes due to the increase in the level of obesity and other related factors worldwide (Jeffery et al., 2003).

## 1.2 PREVALEANCE OF DIABETES IN MALAYSIA

There is a growing number of people diagnosed with diabetes, but with the growing number of people diagnosed with diabetes, Malaysia is not spared of this phenomenon, as prevalence stands at 14.9% of adult population. Adequate blood glucose control is vital in diabetes management to prevent complications, rapid changes, with the prospect of a changing health scenario, has led Malaysians to be affected by western health problems (Jeffery et al., 2003 & Ismail et al., 2001). As of

2001, study noted that 10% of the Malaysian population were diagnosed diabetics (Ismail et al., 2001). Studies also found that the majority of diabetics did not have their disease under control, where 61.1% of patients had HbA1c greater than 8.0%. The studies found that 87.1% of them were hypertensive, 63.5% had a family history of diabetes, 51.6% were not on any hypertensive medications, and 37.2% had microalbuminuria (Ismail et al., 2001).

Complications of diabetes have caused Malaysia to be ranked as number one in kidney failure. A great concern as it implied that diabetes was not well controlled in Malaysia. The number of Malaysians suffering from end-stage renal failure has increased more than 56-fold between 1980 and 2007 (from 43 in 1980 to 12.000) there could also be more unregistered cases. To tackle this problem, the Ministry of Health (MOH) has embarked on a new strategy to reduce these alarming figures through educating, counselling and getting doctors' cooperation. Among these measures are applying new knowledge and innovations gained from multiple sources and disciplines to ensure much needed changes in the health care system for example, the use of genetic informatics and role of genetic markers in risk of chronic diseases of diabetes and cardiovascular diseases, implementing new strategies and understanding behaviour of patients to achieve better health (Merican, 2008).

Despite various measures taken, there is still lack of diabetic control among people with diabetes in Malaysia and there is a need to understand clearly regarding this situation and hopefully the Natural product diabetic research could provide a significant contribution for alliviating such dreadening disease from our societies.

The field of herbal medicines research has been gaining significant importance in the last few decades and the demand to use natural products in the treatment of diabetes is increasing worldwide. The available literature shows that there are more

than 400 plant species showing antidiabetic activity (Rai, 1995). Therefore it is prudent to look for altenatives in exploring herbal medicines for diabetes as well. Although, herbal medicines have long been used effectively in treating diseases in many countries of the world, the mechanism of most of the herbals used has not been clearly defined. Many traditional plant treatments were used for treating diabetes, but most of the evidence for their scientific effects is anecdotal (Prasad et al., 2009). Hence traditional antidiabetic plants might provide new oral hypoglycemic compounds, which can counter the high cost and poor availability of the current medicines/ present day drugs for many rural populations in developing countries.

### 1.3 *TETRACERA SCANDENS* (LINN.) (*T. SCANDENS* L.):

(Family: Dilleniaceae) (local Malaysian name- mempelas kasar) is a climbing vine growing from 3 to 5 meters or more in length and grows widely in India, southern China, Indonesia, Myanmar, Philippines, Thailand, Vietnam and Malaysia.

Different parts (leaves, stems and roots) of *T. scandens* L. are used in folk remedies by various indigenous people in different countries for the treatment of rheumatism, lowering blood pressure, inflammatory diseases, hepatitis, internal pains, urinary disorders, dysentery, child birth, sore throat, gout and diabetes related infirmities (Tawan, 2001; Werner, 2002; Nguyen et al., 2004; Purkayastha et al., 2007; Myung et al., 2009).

The polar extracts of *T. scandens* L. have been reported to exhibit potential therapeutic Xanthenes Oxidize (XO) inhibitory activity in a concentration-dependent manner *in vitro* (Nguyen et al., 2004). Isoflavonoids isolated from the leaves of *T. scan dens* L., have been shown to exert significant glucose uptake effect in basal and insulin-stimulated L6 Myotubes *in vitro* suggesting its potential in the management of

diabetes (Myung et al., 2009). However, no scientific report of this plant *in vivo* has ever been recorded or mentioned in literature showing an antidiabetic efficacy of the leaves of *T. scandens* L. with respect to confirm its utility in folkloric medicine by the local herbalists in Malaysia.

## 1.4 GENERAL OBJECTIVES

- To scientifically prove the traditional claims of the leaves of *T. scandens* L. in the treatment of diabetes related infirmities in Malaysia using animal models *(in vivo)*.
- To investigate phytoconstituents of the leaves of *T. scandens* L.

## 1.5 SPECIFIC OBJECTIVES

- To prepare polar and non-polar extracts of the leaves of *T. scandens* L.
- To investigate antidiabetic activity of polar and non-polar extracts *in vivo.*
- To isolate and characterize the structures of phytoconstituents present in polar and non-polar extracts of the leaves of *T. scandens* L.
- Establish a scientific basis for the therapeutic use of *T. scandens* L.

## 1.6 RESEARCH HYPOTHESIS

- *T. scandens* L. might contain therapeutically active principles which could play a lead role towards the management of diabetes.
- Active principles could be isolated and their structures could be characterized through chemical and spectral analysis.