UNIVERSITI PUTRA MALAYSIA

EVALUATION OF ANTIDIABETIC PROPERTIES OF MORINGA OLEIFERA LAM. LEAVES USING IN VITRO MODEL

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

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April 2015

Chairperson: Professor Amin Ismail, Ph.D

Faculty: Medicine and Health Sciences

Diabetes mellitus is a chronic condition affecting million of people worldwide including Malaysia. 347 million of people worldwide suffering diabetes and National Health and Morbidity Survey in 2011 showed that 20.8 % of Malaysian population suffering diabetes. Even though plenty of antidiabetic drug are available, it is still remain the major worldwide health problems, which possibly due to the current drug adverse side effect and its poor clinical efficacy. Continuous efforts are needed in searching for new, safe and efficacious antidiabetic drug. *Moringa oleifera* is one of the common medicinal plant uses in folk medicine. Traditionally, it has been claimed to possess antidiabetic property. The study was carried out to authenticate the claimed antidiabetic property and its possible mode of actions. In this study, antioxidant capacity and antidiabetic evaluations on hot aqueous *M. oleifera* leaves extract using *in vitro* model were done. Phytochemical study on *M. oleifera* indicated the presence of polyphenols, with total phenolics content was found to be 3550 ± 100 mg gallic acid equivalent per 100 g dry matter basis. It was shown from β-carotene bleaching assay that *M. oleifera* preventing degradation of β-carotene by the peroxyl radicals and exhibited 36% antioxidant capacity, whereas 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay exhibited that IC_{50} for hot aqueous *M. oleifera* was 0.32 mg/ml, suggesting that the extract potentially possesses free radical scavenging ability. Toxicity evaluation by cell viability assay showed that *M. oleifera* did not cytotoxic to BRIN-BD11, 3T3F442A adipocytes, L6 myotubes and Chang liver cells. Studies on antidiabetic mechanism had shown that hot aqueous *M. oleifera* stimulated insulin secretion from pancreatic β-cells significantly ($p< 0.01$). It had been found that maximal insulin secretion ability of *M. oleifera* was 4.66 fold higher than glibenclamide. *M. oleifera* enhanced basal and insulin-mediated glucose uptake into adipocytes, muscles and liver cells. Insulin mimetic property was observed in 3T3F442A adipocytes cells whereas insulin sensitizing property and synergistic effect with insulin property were observed in all cells tested. *In vitro* α-glucosidase inhibition activity had shown that hot aqueous *M. oleifera* significantly inhibited rat intestine sucrase activity with IC_{50} value of 977.24 µg/ml and exhibited competitive-type

inhibition mechanism with Vmax value of 0.283 ± 0.003 mM/mg protein/min. This study showed that *M. oleifera* possesses antidiabetic properties as claimed by the folk medicine practitioners. Coupled with its antioxidant properties, it is suggested that the plant has a potential to be developed as a new plant-derived oral antidiabetic agent.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

PENILAIAN SIFAT-SIFAT ANTIDIABETIK TERHADAP DAUN *MORINGA OLEIFERA LAM.* MENGGUNAKAN MODEL *IN VITRO*.

Oleh

MUHAMMMAD HANAFFI BIN MOHAMAD MOKHTAR

April 2015

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Diabetis melitus merupakan suatu penyakit kronik yang dihidapi di seluruh dunia termasuklah di Malaysia. 347 juta manusia di seluruh dunia mengidap diabetis dan Survei Kesihatan dan Morbiditi Kebangsaan (NHMS) pada 2011 menunjukkan bahawa 20.8% daripada populasi di Malaysia mengidap diabetis. Walaupun ubat antidiabetik boleh didapati namun penyakit ini masih lagi menjadi masalah kepada dunia. Ini mungkin disebabkan oleh kebanyakkan ubat antidiabetik mempunyai kesan sampingan dan mempunyai tahap keberkesanan klinikal yang kurang. Satu tindakan yang berterusan untuk mencari ubat antidiabetik baru yang lebih selamat dan berkesan haruslah diteruskan. *Moringa oleifera* merupakan suatu tumbuhan yang lazim digunakan dalam perubatan turun-temurun. Secara tradisional, tumbuhan ini didakwa mempunyai sifat antidiabetik. Kajian ini dijalankan untuk mengesahkan dakwaan yang menyatakan bahawa ekstrak akuas panas daun *M. oleifera* mempunyai sifat antidiabetik termasuklah menjalankan kajian keatas mod antidiabetik yang mungkin. Kajian yang dijalankan melibatkan kajian terhadap kapasiti antioksidan dan penilaian-penilaian antidiabetik terhadap ekstrak akuas panas *M. oleifera* menggunakan model *in vitro*. Kajian fitokimia ke atas *M. oleifera* menunjukkan kehadiran polifenol dengan nilai kandungan fenolik keseluruhan ialah 3550 ± 100 mg asid galik setara per 100 g berat kering *M. oleifera*. Dari kajian pelunturan β-karoten, didapati *M. oleifera* dapat mengelakkan pelunturan β-karoten oleh radikal peroksil dan memberikan 36% kapasiti antioksidan. Dari ujikaji kesan pemburuan terhadap radikal 2,2-diphenyl-2-picrylhydrazyl (DPPH), ujikaji menunjukkan bahawa nilai IC_{50} ekstrak ialah 0.32 mg/ml dan ini menyarankan bahawa ekstrak berpotensi dan berkemampuan menurunkan kandungan radikal bebas. Kajian toksisiti sel dengan kaedah asei kebolehhidupan sel menunjukkan bahawa ekstrak akuas panas *M. oleifera* tidak mempunyai kesan toksik terhadap sel-β pankreas, sel adiposit, sel otot dan sel hati. Kajian mekanisma antidiabetik menunjukkan bahawa ekstrak *M. oleifera* merangsang secara signifikan perembesan insulin daripada sel-β pankreas ($p<0.01$) dan didapati bahawa kemampuan maksimum ekstrak *M. oleifera* merangsang perembesan insulin adalah 4.66 kali ganda lebih tinggi jika dibandingkan dengan glibenklamid. Ekstrak *M.*

oleifera juga didapati meningkatkan pengambilan glukosa basal dan pengambilan glukosa yang diperantarakan-insulin ke dalam sel-sel adipos, otot dan hati. Ciri seperti-insulin diperhatikan pada sel adiposit manakala ciri sensitif-insulin dan ciri kesan sinergistik dengan insulin diperhatikan pada semua sel yg terlibat dalam kajian ini. Dari kajian perencatan aktiviti α-glukosidase secara *in vitro* didapati ekstrak *M. oleifera* merencat aktiviti enzim sukrase usus tikus secara signifikan dengan nilai IC_{50} bersamaan 977.24 µg/ml dan mempamerkan mekanisma perencatan bersaing dengan nilai Vmax bersamaan 0.283 ± 0.003 mM/mg protein/min. Kajian ini menunjukkan *M. oleifera* memiliki sifat-sifat antidiabetik sebagaimana didakwa oleh pengamal pengubatan turun-temurun. Digandingkan dengan sifat-sifat antioksidan yang dimilikinya, *M. oleifera* didapati berpotensi untuk dibangunkan sebagai agen antidiabetik.

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I certify that a Thesis Examination Committee has met on 14 April 2015 to conduct the final examination of Muhammad Hanaffi bin Mohamad Mokhtar on his thesis entitled "Evaluation of Antidiabetic Properties of *Moringa oleifera* Lam. Leaves using *In Vitro* Model" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

%	Percentage
µg	microgram
µl	microlitre
µM	micromolar
µm	micrometer
^{3}H	Tritium
AMP	Adenosine monophosphate
ANOVA	Analysis of Variance
ATCC	American type cell culture
ATP	Adenosine triphosphate
BHT	Butylated hydroxytoluene
BSA	Bovine serum albumin
$CaCl_2$	Calcium chloride
cAMP	Cyclic adenosine monophosphate
Ci	Curie
cm^3	centimeter cubic
CO_2	Carbon dioxide
Da	Dalton
DMEM	Dulbecco's modified eagle's medium
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
ELISA	Enzyme-linked immunosorbent assay
FBG	Fasting blood glucose
FBS	Foetal bovine serum
g	gram
GLUT	Glucose transporter
H_2O	Water
H_2SO_4	Sulfuric acid
HbA_{1C}	Glycosylated haemoglobin
HCl	Hydrochloric acid
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

IC_{50}	Half maximal inhibitory concentration
IDDM	Insulin Dependent Diabetes Mellitus
IL	Interleukin
IR	Insulin receptor
IRS	Insulin receptor substrate
K	Potassium
K^+_{ATP} channel	ATP-sensitive potassium channel
KCl	Potassium chloride
kDa	kilodalton
kg	kilogram
KH_2PO_4	Potassium dihydrogen phosphate
KRB	Krebs ringer bicarbonate buffer
L	litre
LLT	Low level tritium
M	Molar
m	meter
MARDI	Malaysian Agricultural Research and Development Institute
mg	milligram
$MgSO_4$	Magnesium sulphate
ml	mililitre
mM	milimolar
mm	millimeter
MTT	3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide
NaCl	Sodium chloride
NAD	Nicotinamide adenine dinucleotide
$NaHCO_3$	Sodium hydrogen carbonates
NaOH	Sodium hydroxide
NCD	Non-communicable disease
NIDDM	Non-insulin dependent diabetes mellitus
^oC	Degree Celsius
OD	Optical density
OGTT	Oral glucose tolerance test
PBS	Phosphate buffer saline

PI3-K	Phosphatidylinositol 3-kinase
rpm	Round per minute
RPMI	Roswell Park Memorial Institute
SDS	Sodium dodecyl sulphate
STZ	Streptozotocin
SUs	Sulfonylurea
TCA	Tricarboxylic acid cycle
TG	Triglyceride
TMB	3,3',5,5' tetramethylbenzidine
TNF	Tumor necrosis factor
TRIS	2-amino-2-hydroxymethyl-propane-1,3-diol
TZDs	Thiazolidinediones
UPM	Universiti Putra Malaysia
USA	United State of America
v/v	volume per volume
VDCC	Voltage-dependent calcium channel
VLDL	Very low density lipoprotein
WHO	World Health Organization
α	Alpha
β	Beta
γ	Gamma

CHAPTER 1

INTRODUCTION

1.1 Background of study

Diabetes mellitus is a disease which characterizes by persistent hyperglycemia in fasting and/or postprandial state with disturbance in carbohydrate, fat and protein metabolism which resulting from the defects of insulin secretion or insulin action or combination of these two factors (Alberti and Zimmet, 1998). Among the symptoms including polydipsia, polyphagia, polyuria, pruritus and unexpected weight loss (B. K. Rao et al., 2001).

As in October 2013, 347 million people worldwide suffering diabetes and in 2004 WHO had estimated that 3.4 million people died from the consequences of high fasting blood sugar. More than 80% of diabetes deaths occur in low and middle income countries. WHO projects that diabetes will be the 7th leading cause of death in 2030 (WHO, 2013). It will be 42% increase from 51 to 72 million diabetic patients in the developed countries and 170% increase from 84 to 228 million in developing countries. By the year 2025, over than 75% diabetic patients are from developing countries (Alberti et al., 2007).

Diabetes is a leading cause of stroke, heart disease, leg amputation, blindness and kidney failure in Malaysia. First National Health and Morbidity Survey (NHMS) in 1986 reported that the prevalence of diabetes among adults aged 30 years and below was 6.3%, rising to 8.3% in second NHMS conducted 10 years later in 1996 and reached 14.9% in third NHMS in 2006. The most recent NHMS (2011) showed that diabetes prevalence was 15.2% which exhibited that approximately about one in five adults or 2.6 million Malaysians now suffer from diabetes (Ministry of Health Malaysia). It is believed that for every one person diagnose with diabetes, there will be at least another one who is remain undiagnosed, which mean another 2.6 million of Malaysians could be suffer from diabetes (Stanley Liew, 2013). The rising trend in the prevalence of diabetes is alarming for Malaysian and could possibly due to the growth of population, aging, urbanization, changes in dietary habit, obesity and sedentary lifestyle (Chehade and Mooradian, 2000; Zaini, 2000; Letchuman et al., 2010). It also has been found that age, ethnic, regional and racial differences have a role for the diabetic incident in heterogeneous populations within the same area (Matthaei et al., 2000; Jacobsen et al., 2009).

The rate of diabetes complications and its associated diseases among diabetes patient are significantly high. The prevalence of diabetes complications such as neuropathy, retinopathy and albuminuria were 30.1%, 23.5% and 22.9% respectively. Poor glycaemic control is the reason for the high complication rates (Ooyub et al., 2004). Malaysia have taken steps through Ministry of Health (MOH) to improve the management and care of diabetic patients in clinics and hospitals including setting up

dedicated centre, the Diabetes Resource Centre in order to train more educators for diabetic nurse and implementing standardized follow-up protocols. Follow up on 2003 found that prevalence of neuropathy, retinopathy and albuminuria reduced to 19.0%, 11.1% and 15.7% respectively. The steps taken by MOH were beneficial even though far from satisfactory. Diabetic patients admitted for diabetic treatment had increased from 19629 cases in 1991 to 30661 cases in 2001 which showed 56% increases in 10 years. According to Ooyub et al. (2004), mortality rates associated with diabetes increased from 254 deaths in 1991 to 380 death in 2001. With all this indications that diabetes had become the major problems and more proactive efforts are needed in preventing and controlling this chronic diseases in Malaysia (Mafauzy, 2004).

The primary target in diabetes treatment is to ensure that the glucose in the blood is within its normal range, which in turn may lower the diabetes complications. Several therapeutic approaches are available in controlling the diabetes, including doing regular exercise, changes of diet and by taking oral antidiabetic drugs. The oral antidiabetic drugs are categorized by their mechanism of actions such as thiazolidinenediones, sulfonylureas, biguanides, meglitinides, α-glucosidase, amylin synthetic derivatives, D-phenylalanine derivatives and incretin mimetics. There is some known issues regarding the limitations of the conventional antidiabetic drugs such as undesirable adverse effect, lacking extensive clinical efficacy and individual variability. In addition, another constrains such as the unavailability and unaffordability of the conventional antidiabetic drugs to the citizen in the remote area in third world and developing countries still remain indefinite. Due to the limitations and constrains mentioned above, there is a need for the continuation in searching for a new antidabetic agents which should be more safer, efficacious and cheaper.

Recent trends have shown that the uses of alternative therapy (including traditional medicine and herbal medicine) in dealing with diabetes mellitus area growing and become more favorable when compared to conventional antidiabetic drug. 343 plants are reported possessed blood glucose lowering effect (Atta-ur-Rahman and Zaman, 1989). Herbal medicine either in its natural state or as a source of new pharmaceutical have shown healing property and several studies showed that herbal medicine can be considered safe for human use, thus it have been widely explored in searching for new antidiabetic agent. In addition, herbal medicine is more affordable compared to conventional antidiabetic drug, thus it is more preferred by vast majority of residents in developing countries (Zhang, 2004).

M. oleifera, locally known as Murunggai is a common plant in Malaysia especially in Indian community. It has been claimed to possess antidiabetic property. The plant has shown therapeutic values such as antidiabetic, anti-inflammatory and antioxidant properties (Adisakwattana and Chanathong, 2011). Despite such claims, scientific study to evaluate its effectiveness in lowering the blood glucose and its possible antidiabetic mechanism are still lacking. Evaluation of *M. oleifera* properties (antioxidant capacity and cell viability studies), evaluation of insulin secretion activity, evaluation of glucose uptake activity and effect of *M. oleifera* on rat intestine α-glucosidase (sucrase) activity had been conducted in this study.

1.2 Problem statement

The primary target for diabetes treatment is to maintain the blood glucose in its normal range thus may lower the risks for diabetes complications. Patient with Type 1 diabetes mellitus had limited treatment, which is relying on insulin therapy for survival (Kirchheiner and Brockmoller, 2005). There are different types of insulin analogues and categorized according to the times and duration of action (Chehade and Mooradian, 2000). For Type 2 diabetes mellitus, there is number of therapeutic approaches in controlling the hyperglycemia in patients including changes of diet, regular exercise and uses of oral antidiabetic drugs. The oral antidiabetic drugs are categorized based on its mechanisms such as biguanides, α-glucosidase, sulfonylureas, meglitinides, thiazolidinenediones, D-phenylalanine derivatives, incretin mimetics and amylin synthetic derivatives. These drugs should be used based on the dynamic pathophysiologically abnormalities of the disease (Chehade and Mooradian, 2000; Babu et al., 2007).

Diabetes mellitus remains as a major global health problem even though there are plenty of antidiabetic drugs available on the market. This could be possibly due to the limitation of the conventional antidiabetic drug such as undesirable adverse effect, lack of clinical efficacy and individual variability (in terms of pharmacokinetic). The other constrains are the unavailability and unaffordability for the citizens in the remote area such as in third world and developing countries. Issues such as difficulties to get these drugs and its high cost in these areas are significant (Chiasson et al., 1994; Andayani and Imaningsih, 2007).

The safety of oral antidiabetic drugs should be taken seriously as it has been reported that some of these drugs are associated with various adverse effects, which in turn could offset the benefit of the drugs. As an example, looking at the drug in the group of sulfonylureas, it has been reported to cause hypoglycemia and weight gain (Chehade and Mooradian, 2000) whereas metformin are associated with nausea, diarrhea, gastrointestinal discomfort and anorexia (Odawara et al., 1997; Charpentier et al., 2000). The uses of meglitinide analogues have been found associated with hypoglycemia (Chehade and Mooradian, 2000) meanwhile α-glucosidase inhibitors, which lowered the blood glucose by inhibiting α-glucosidase enzyme in small intestine reported to cause disturbances in gastrointestinal tract (Coniff and Krol, 1997; Watkins and Whitcomb, 1998), hepatotoxicity (Chilcott et al., 2001), renal tumors (Andayani and Imaningsih, 2007) and lethal ileus (Odawara et al., 1997). Meanwhile the uses of drugs in the group of thiazolidinediones can cause liver injury, weight gain, fluid retention and anemia (Odawara et al., 1997; Watkins and Whitcomb, 1998).

Some drugs are found not effective when used as monotheraphy but effective when the drugs are combined with other oral antidiabetic drugs. As an example, pioglatizone are not effective when use solely by itself but effective when used as an adjunct to metformin or sulfonylurea (Chilcott et al., 2001). Another examples are rosiglitazone, the efficacy of rosiglitazone found to be better when used as add-on therapy with traditional antidiabetic drug (Boucher et al., 2003). Among individuals lies variability in the pharmacokinetics of the antidiabetic drugs, Kirchheiner and Brockmoller (2005)

shown that the intervariability are contributed by the genetic factors which included differences in drug transport, bioavailability, metabolism, as well as drug action which in turn may affect clinical outcomes and risk of adverse effects between patients.

Due to the limitations of conventional and current antidiabetic drugs as mentioned above, continuation in searching for new antidiabetic agents which are more safer, efficacious and cheaper should be continued. The limitations of the modern antidiabetic drugs have been among the factors why peoples turned to alternative therapy for treatment and maintenance of diabetes mellitus.

1.3 Significance of study

The uses of alternative therapy in dealing with prevention, treatment and maintenance of diabetes mellitus are growing and become favorable compared to conventional antidiabetic drugs. This is due to the limitations that associated with conventional antidiabetic drugs. Alternative therapy, as well as traditional medicine, played an important role in developing countries health care system (Zhang, 2004; Dieye et al., 2008). Naturopathy is the most common alternative therapy for treatment of diabetes (Bradley and Oberg, 2006) which also includes homeopathy (Roy, 2009), massage, dietary supplements, acupuncture (Yeh et al., 2003) and herbal medicines (Atta-ur-Rahman and Zaman, 1989; Al-Rowais, 2002; Balde et al., 2006).

It has been estimated that 80-85% of population in developed and developing countries rely on traditional medicines to fulfill their health care needs and the major part of traditional therapy involved the use of plant extract or the plant active compounds (Tomlinson et al., 1998; Ignacimuthu et al., 2006; Dieye et al., 2008).

Atta-ur-Rahman and Zaman (1989) reported that more than 343 plant possessed hypoglycemic properties. Herbal medicines have shown to have healing properties, either in its natural state or as sources of new pharmaceutical. In addition, formulation of herbal medicines can be considered less toxic compared to the formulation of pharmaceutical agent (Elvin-Lewis, 2001). Babu et al. (2007) had found that we can expect the herbal medicines have similar degree of efficacy when compared to conventional antidiabetic drugs. Several studies have shown that herbal medicines can be considered as safe for human use and therefore it has been widely explored in searching for new antidiabetic agents (Li et al., 2005). In term of cost, herbal medicines are more affordable when compared to conventional antidiabetic drugs thus are more preferred by the vast majority of residents in developing countries (Zhang, 2004). Herbal medicines have been used as modern drugs substitution in remote areas where the modern drugs are not easily accessible.

Plants such as *Ortosiphon stamineus*, *Averrhoa bilimbi*, *Tinospora crispa*, *Andrographis paniculata*, and *Gynura procumbens* have been reported through scientific studies showing antidiabetic activities. These plants have been used extensively in folk medicines as remedy for diabetes mellitus in Malaysia (Mafauzy,

2004). Beside of these plants, there is common plant which has been claimed to possess antidiabetic property,namely *Moringa oleifera*. *M. oleifera* or locally known as Murunggai is well known plant with therapeutic values especially in Indian community in Malaysia and worldwide. *M. oleifera* can be found grown in the tropics and sub-tropics of Asia and Africa (Iqbal and Bhanger, 2006) and it is the most widely cultivated species of Moringaceae in Sri Lanka, Pakistan, India, North-eastern and South-eastern Africa, Thailand, Bangladesh and Arabia. The plant has shown therapeutic values such as in prevention and treatment of diseases such as anti-diabetic, anti-inflammatory, anti-microbial and antioxidant properties (Adisakwattana and Chanathong, 2011). Recent studies have shown that methanolic fraction of the leaves possesses anti-ulcer whereas pressed juice of the leaves shown strong antibacterial activity against *E. coli*, *Bacillus subtilis* and *Micrococcus pyogenes var aureus* (Siddhuraju and Becker, 2003). Estrella et al. (2000) reported that *M. oleifera* leaves increase breast milk production among mother with preterm infants. Hot aqueous of *M. oleifera* leaves extract also reported to have strong antioxidant property (Chumark et al., 2008). The antioxidant property was reported probably correlated with the presence of alkaloids, moringine (Kirtikar and Basu, 1975), flavonoids, tocopherols, quercetin, vitamin C, carotenoids, kaempferol (Laandrault et al., 2001), glycosides and benzylisothiocyanate with its derivatives (Das et al., 1958).

It is important that foods which contain phytochemicals are not consumed in isolated and purified form but in combination with other phytochemicals and food components, by this way the consumption of these plants can serve as a vital role for dietary disease-preventive food components (Dillard and German, 2000).
Based on ethnobotanical approaches, *M. oleifera* has been claimed traditionally to possess antidiabetic properties (Dieye et al., 2008). Despite such claims and widespread use, however, the scientific studies to evaluate its effectiveness in preventing diabetes risk factor and its possible antidiabetic mechanism are still lacking. *In vivo* studies have shown that *M. oleifera* exhibited antidiabetic properties in normal and diabetic rats (Ndong et al., 2007; Jaiswal et al., 2009). Therefore, in order to understand the mechanisms underlies the antidiabetic actions of *M. oleifera*, *in vitro* studies have been conducted, specifically evaluation of insulin secretion activity, evaluation of glucose uptake activity and effect of *M. oleifera* on rat intestine α-glucosidase (sucrase) activity.

Hot aqueous extract was used in evaluating the antidiabetic property of *M. oleifera* in order to emulate the traditional practice of folk medicine practitioner in prescribing the extract as antidiabetic medication. The common practice is to boil the *M. oleifera* leaves in the water before it is consumed as herbal remedy. This present study sought to establish the scientific validity for the antidiabetic activity of hot aqueous *M. oleifera* leaves extract as claimed by the folk medicine practitioners.

1.4 Objectives of study

General Objective

To evaluate antidiabetic activity of hot aqueous *M. oleifera* leaves extract as claimed by the folk medicine practitioners through *in vitro* model.

Specific Objectives

1. To determine antioxidant capacity of *M. oleifera* using total phenolic assay, β-carotene bleaching assay and 2,2-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay.

2. To evaluate *M. oleifera* on cell viability properties for cytotoxicity evaluation on BRIN-BD11 cells, 3T3-F442A adipocytes, L6 myotubes and Chang liver cells.

3. To elucidate possible antidiabetic mechanisms of *M. oleifera*, specifically stimulation of pancreas insulin, enhancement of glucose uptake into muscle, adipocytes and liver cells and inhibition of α-glucosidase enzyme in small intestine.

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