Review Article

Flavonoid from *Moringa oleifera* leaves revisited: A review article on *in vitro*, *in vivo*, and *in silico* studies of antidiabetic insulin-resistant activity

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Abstract

Diabetes mellitus (DM) occurs when the body experiences insulin deficiency or is unable to use insulin appropriately, which increases the blood glucose levels over the threshold. *Moringa oleifera* leaf is a widely used and scientifically proven herbal medicine to treat DM. The demand for the development of new drugs has prompted *in vitro*, *in vivo*, and *in silico* studies of antidiabetic insulin-resistant activity. This study aims to conduct a comprehensive study of the types of flavonoid and nonflavonoid compounds that have antidiabetic activity in insulin resistance mellitus using *in vitro*, *in vivo*, and *in silico* approaches. The literature review was conducted in accordance with the offered reporting items for systematic review. Major bibliographic databases, i.e. Scopus, PubMed, and DOAJ, covering original articles about the aforementioned issues between January 1, 2011 and December 31, 2021 were used. In this study, 274 articles were retrieved, of which 4 were duplicates, and after the titles were read, only 108 were left for analysis. After the abstract screening, 32 articles were eligible for the literature review. The results exhibit that flavonoids, including quercetin and kaempferol, and nonflavonoids, including anthraquinone, cytogluside (glycoside), hemlock tannin, phenolic steroid, and 2-phenylchromenylium (anthocyanins), have potential insulin-resistant antidiabetic activity *in vitro*, *in vivo*, and *in silico*. This has broadened the research into the development of new drugs.

Key words: Antidiabetic, flavonoid, insulin-resistant, kaempferol, *Moringa oleifera*, quercetin

INTRODUCTION

Blood glucose levels rise as a result of diabetes mellitus (DM), a condition, in which the body is unable to make enough insulin or use it efficiently. DM is a complex long-term systemic disease and is accompanied by metabolic problems such as hyperglycemia, hyperinsulinemia, and hypertriglyceridemia.[1] DM is linked to long-term difficulties and affects approximately 537 million individuals between the ages of 20 and 79. In 2030 and 2045, respectively, this number is projected to reach 643 million and 783 million. The case number is a health burden because it causes many organ injuries and various complications. Hence, it causes

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not only a high mortality rate but also a significant decrease in patients' quality of life.

The use of natural ingredients is expected to solve one of the health problems while strengthening the community's economy. Some scientific evidence shows that compounds from natural ingredients can be therapeutic agents for insulin-resistant diabetic drug discovery with relatively low toxicity and no adverse side effects. An increasing number of natural substances have been identified as having antidiabetic characteristics that are resistant to insulin recently, and several efforts have been made to understand the underlying mechanisms.[1] For example, flavonoid compounds, which are bioactive compounds in *Moringa oleifera* leaves, have demonstrated insulin-resistant antidiabetic activity. Quercetin (±50% of the total flavonoids)[2] and kaempferol [Figure 1], a flavonol bioactive compound, are the main flavonoids with a similar chemical structure; thus, both compounds have similar biological activities and can work synergically.[3]

In addition, according to *in vitro* and *in vivo* studies, these main compounds can lower blood glucose levels by boosting insulin production, improving its sensitivity, and reducing amylase glucosidase activity [Figure 2].[4] This was also proved by quercetin being able to increase sodium–glucose cotransporter-2 (SGLT-2) receptors and kaempferol being able to increase the glucose transporter 4 (GLUT4) transporters in *in silico* studies. Further compounds in *M. oleifera* leaves, with a predominance of phenolic derivatives, have been reported to contribute to the antidiabetic activity in another *in silico* assay. The cocrystallized ligands of α-amylase, α-glucosidase, and dipeptidyl peptidase-4 (DPP-4), which are also identified *in silico*, have antidiabetic activities against α-amylase, α-glucosidase, and DPP-4.[5]

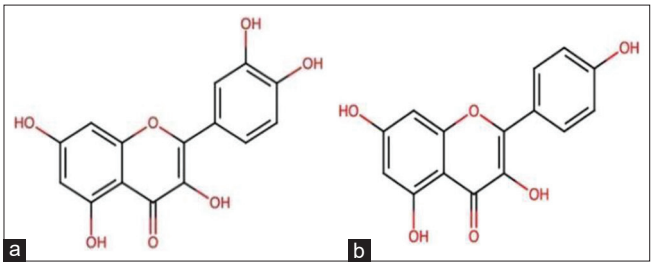

Figure 1: Structures of major flavonoids found in *Moringa oleifera* L. (a) Quercetin and (b) Kaempferol

The novelty of this literature review to give a thorough overview of *in vitro*, *in vivo*, and *in silico* approaches to the study of flavonoid and nonflavonoid compounds of *M. oleifera* in the evaluation of new drugs with antidiabetic insulin-resistant activity and collection of data for subsequent clinical trials involving humans. The recent literature review regarding *M. oleifera* not highlighting an overview of *in vitro*, *in vivo*, and *in silico* approaches to the study of flavonoid and nonflavonoid compounds of *M. oleifera* in the evaluation of new drugs with antidiabetic insulin-resistant activity.[6] The flowchart for the selection of articles in the literature review using the PRISMA methodology [Supplementary Material 1].

BIOACTIVE PHYTOCHEMICALS OF *MORINGA OLEIFERA* LEAVES

The leaves of *M. oleifera* have been shown to include carotenoids, alkaloids, flavonoids, glycosides, anthocyanins, anthraquinones, saponins, steroids, tannins, and terpenoids.[7,8] The chemical components of *M. oleifera*'s leaves play a vital role in several pharmacological processes that address diabetic problems and risk factors,[9] such as alkaloid compounds, glucosinolates, and isothiocyanates as anticancer;[10] phenolic acid compounds

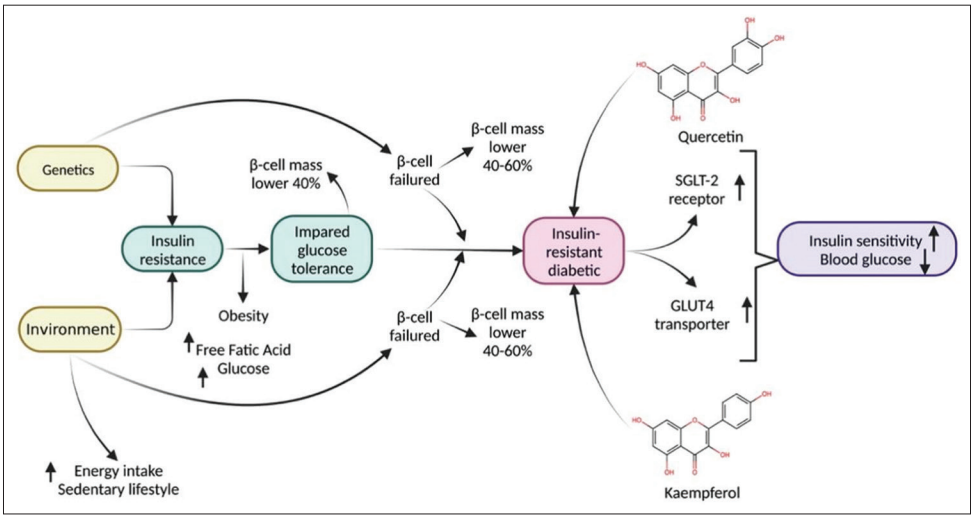

Figure 2: Pathogenesis of insulin-resistant diabetic and mechanism of quercetin and kaempferol in increasing insulin sensitivity and decreasing blood glucose. SGLT-2: Sodium-glucose co-transporter-2, GLUT4: Glucose transporter 4

and isothiocyanates as antibacterial;[11] phenolic acids, tannins, steroids, saponins, alkaloids, and flavonoids as analgesic and wound healing agents.[12,13] Furthermore, glucosinolates and isothiocyanates have antioxidant activities;[14] and anthraquinones, sitogluside (glycosides), tannins, steroids, phenolics, anthocyanins, quercetin, and kaempferol showed antidiabetic and obesity activities.[9] Nevertheless, the leaves are the most commonly used in numerous traditional medications and human health care; therefore, they are potentially developed as modern phytopharmaceuticals [Figure 3].[15]

Studies have reported that the extract of *M. oleifera* leaves contains several active compounds, including phenols, flavonoids, glucosides, and alkaloids. Factors such as the harvesting intervals at 30, 45, and 60 days, extraction technique, and solvents employed to affect the concentrations of the active components in *M. oleifera* leaves.[16-20] Due to the more active biosynthetic processes in the cells, the chemical content of *M. oleifera* leaves rises as the plant ages. For this investigation, fresh leaves of *M. oleifera* were collected at varied harvest periods. Fresh leaves were taken 30, 45, and 60 days after the trees were trimmed. Fresh leaves picked at age >60 days exhibited large quantities of phenols and flavonoids [Supplementary Material 2].[19]

Subsequently, the types of solvent and the extraction method used to influence the phytochemical profile of the extracts were examined.[20] Optimized the extraction process of *M. oleifera* leaves using two extraction procedures, i.e. direct maceration and successive maceration, and four other solvents were used, i.e. dichloromethane, ethyl acetate, *n*-butanol, and water. As a result, the direct maceration method and water were observed to yield the greatest phenol and flavonoid contents [Supplementary Material 3].[16]

ROLE OF FLAVONOID AND NONFLAVONOID COMPOUNDS IN *IN VITRO*, *IN VIVO* ASSAY, AND *IN SILICO* APPROACHES

In vitro assay

The extract of *M. oleifera* leaves may be used as a hypoglycemic medication.[21] This hypoglycemic activity was due to α-amylase enzyme inhibition by the methanolic extract (IC_{50} 8.217 ± 0.792 µg/mL) and hexane extract (IC_{50} 9.397 ± 0.298 µg/mL). However, these values were comparable to acarbose as the positive control (IC_{50} 0.036 ± 0.001 µg/mL), although they were slightly weaker than the corresponding control.[22] In addition to inhibiting α-glucosidase (another enzyme playing a role in diabetic pathogenesis) with IC_{50} of 19.36 ± 2.43 µg/mL, this extract inhibited lipase (an enzyme that could be a risk factor of DM) from the pancreas with IC_{50} values of 123.34 ± 3.89 µg/mL. The compounds responsible for this mechanism are flavonoids such as quercetin and kaempferol.[23] Further study on the combination of *M. oleifera* leaves with guava leaf extracts can increase its effectiveness in inhibiting the amylase enzyme compared with acarbose.[24]

In vivo experiments

The *in vivo* test intends to determine the activity of compounds on experimental animals, which were

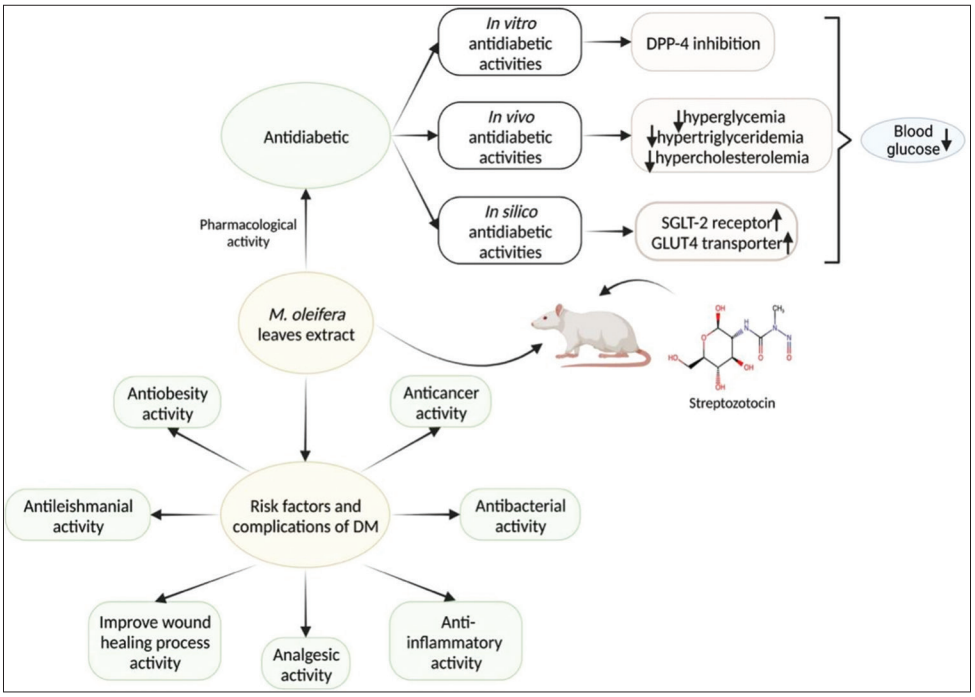

Figure 3: Pharmacological activities of *Moringa oleifera* extract toward some risk factors and diabetes mellitus complications. SGLT-2: Sodium–glucose co-transporter-2, GLUT4: Glucose transporter 4, DPP-4: Dipeptidyl peptidase-4, DM: Diabetes mellitus, *M. oleifera*: *Moringa oleifera*

divided into the test group, the negative control group, and the positive control group, etc., according to the study [Supplementary Material 4].

In diabetic rats and mice, *M. oleifera* leaf extracts significantly boosted the ability of pancreatic cells to secrete insulin.[25-27] This was corroborated by a study that discovered a dose-dependent rise in insulin sensitivity in diabetic rats. Doses of 250 and 500 mg/kg were found to significantly reduce homeostatic model assessment for insulin resistance levels.[28] One of the isolated compounds from *M. oleifera* leaf extracts was fluoropyrazine, which induced significant insulin secretion.[29] Another identified compound was kaempferol, which also induced insulin secretion at 200 mg/kg/day, which was equivalent to 200 g/kg twice daily administration of liraglutide in the control group.[30]

In silico study

The compounds in *M. oleifera* leaves extract were studied for their binding in the mutated diabetes receptor kinase domain in complex with cis-(R)-7-(3-(azetidin-1-ylmethyl) cyclobutyl)-5-(3-((tetrahydro-2H-pyran-2-yl) methoxy) phenyl)-7H-pyrrolo [2, 3-d] pyrimidin-4-amine using molecular docking. It was found that five compounds that met Lipinski's rules including 2-phenylchromenylium (anthocyanin), phenolic steroid, hemlock tannin, sitogluside (glycoside), and anthraquinone [Figure 4], showing some molecular interactions with the receptor via van der Waals interaction.[31]

Docking studies were also carried out using receptor proteins on SGLT-2, GLP-1, and peroxisome proliferator-activated receptor gamma on quercetin and kaempferol by showing strong affinities toward those three receptors. Quercetin exhibited the best affinity toward the SGLT-2 receptor, whereas kaempferol complied with Lipinski's rule of 5, Modern Drug Data Report, and VERBER's rule from a total of four rules that must be met in drug-likeness prediction. Another docking test was conducted using α-glycosidase as the targeted enzyme. Myricitrin, quercetin, and polydatin bound to the main site while interacting with residues ARG442 and GLU411 at the catalytic site.[32] Kaempferol had the highest affinity to the GLUT4 transporter, another protein target in antidiabetic discovery.[33]

Compounds in *M. oleifera* leaf extract evaluated their effects on α-amylase, α-glucosidase, and DPP-4 and found that stevioside had an energy affinity of −6.893 kcal/mol, which approached the cocrystallized ligand of α-amylase with −7.811 kcal/mol. Besides, butyloxycarbonyl oxy-1 was found to show an energy affinity of −5.583 kcal/mol, which was close to the cocrystallized ligand of DPP-4, α-amylase, and α-glucosidase (−6.102 kcal/mol).[5]

CONCLUSION

The antidiabetic insulin-resistant activities observed in *in vitro*, *in vivo*, and *in silico* approaches have proven their applicability in new drug development research and can be used as data for further research in clinical trials involving humans. The evaluated *in vitro*, *in vivo*,

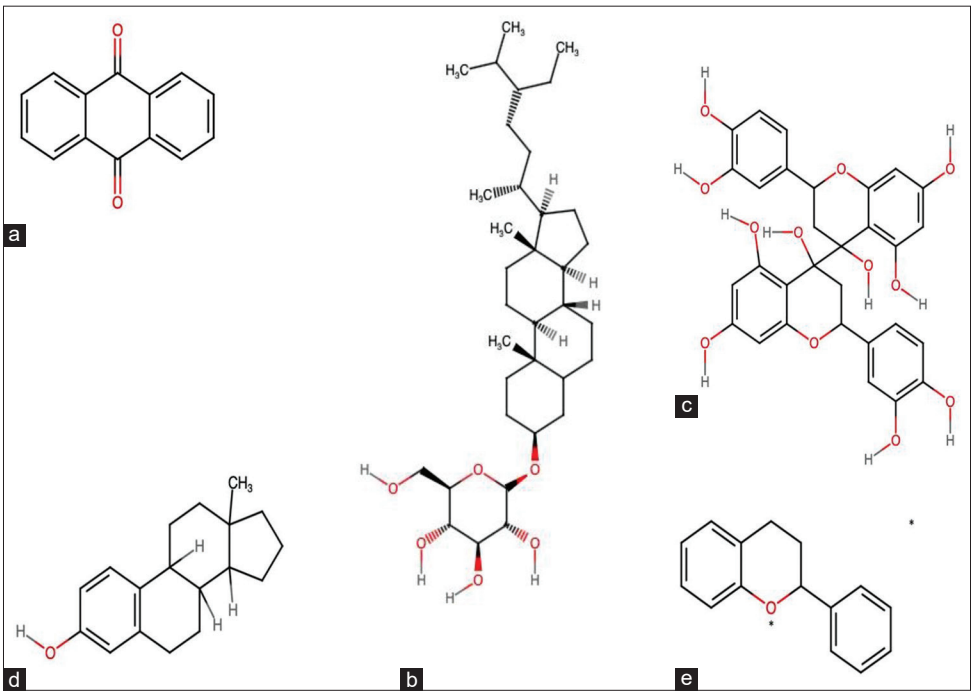

Figure 4: Structures of (a) anthraquinone, (b) sitogluside (glycoside), (c) hemlock tannin, (d) phenolic steroid, and (e) 2-phenylchromenylium (anthocyanin) in *Moringa oleifera* leaves extract

and *in silico* studies have similarities regarding flavonoid compounds, including quercetin and kaempferol, and nonflavonoids, including anthraquinones, sitogluside (glycosides), hemlock tannins, phenolic steroids, and 2-phenylchromenilium (anthocyanin), which have been shown to have insulin-resistant antidiabetic activity. These results can also meet the demand for research needs regarding the development of new drugs.

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Conflicts of interest

There are no conflicts of interest.

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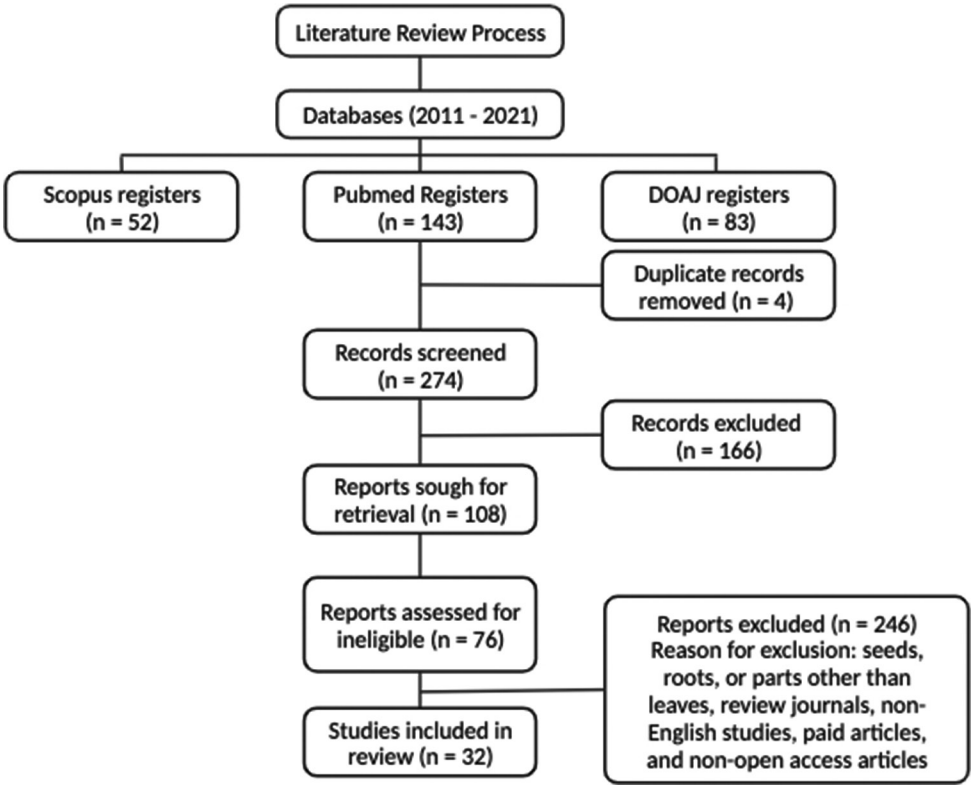

Supplementary Material 1: Flow chart of the literature review process

Supplementary Material 2: Effects of the harvesting time and extraction solvent used on the total phenolic and total flavonoid contents of *Moringa oleifera* leaves

Solvent	Total phenolics (g GAE/100 g DE) (days)			Total flavonoids (g QE/100 g DE) (days)		
	30	45	60	30	45	60
Ethanol	3.32	3.64	3.97	1.40	1.82	1.82
Methanol	3.91	4.02	4.57	0.96	1.12	1.13
Water	3.20	3.67	2.16	0.93	0.92	1.04

DE: Dry extract, GAE: Gallic acid equivalent, QE: Quercetin equivalent

Supplementary Material 3: Effect of the extraction method and solvent on the total phenolics and total flavonoids

Extraction methods	Solvent	Total phenolics (mg GAE/g DE)	Total flavonoids (mg QE/g DE)
Direct maceration	DCM	24.17	11.76
	EtOAc	31.19	20.05
	n-But	38.11	19.45
	Water	101.81	45.57
Successive maceration	DCM	18.19	12.26
	EtOAc	40.29	23.39
	n-But	103.06	41.81
	Water	69.72	22.34

DCM: Dichloromethane, EtOAc: Ethyl acetate, *n*-But: Butanol, DE: Dry extract, GAE: Gallic acid equivalent, QE: Quercetin equivalent

Supplementary Material 4: Recent reports of *Moringa oleifera* leaf extracts studied *in vivo* as an antidiabetic animal model

Samples	Subjects	Methods	Concluding remarks	References
Protein isolate	Male mice, 3 weeks old, from Biocen-UFC progeny stock originating from Switzerland	*In vivo*: Alloxan-induced mice, i.p. doses 150 mg/kg BW, sample doses of 100, 300, and 500 mg/kg BW	Doses of 500 mg/kg BW provide better antidiabetic activity, with a decrease in blood glucose of 34.3%, 60.9%, and 66.4% after 1, 3, and 5 h, respectively. This protein isolate is a promising complementary agent for treating diabetes	Paula *et al.*, 2017b
Ethanol 95% extract	Male Wistar rats weighing 100–120 g	Streptozotocin-induced rats, doses of 40 mg/kg BW, sample doses of 100, 200, and 400 mg/kg BW	Significantly ($P<0.001$) caused a decrease in blood glucose levels, whereas it was not significantly decreasing ($P>0.05$) in mice given normal saline	Anwer *et al.*, 2021
Methanol 80% extract	Adult male Wistar rats weighing approximately 200 g and 250 g, aged 10 weeks and from Stellenbosch, Tygerberg, South Africa	Streptozotocin-induced rats; doses of 55 mg/kg BW, sample doses of 250 mg/kg BW	Plasma glucose levels decreased significantly ($P<0.05$) in diabetic rats after treatment when compared with diabetic control (DM)	Omodanisi *et al.*, 2017
Methanolic extract	Rats (Sprague-Dawley) male (180–200 g)	In vivo: Alloxan 150 mg/kg-induced mice	It reduces blood glucose levels	Saucedo-Pompa *et al.*, 2018
Methanol 80% extract	Male Wistar rats	*In vivo*: Alloxan-induced rats doses of 170 mg/kg, sample doses of 200 mg/kg in 3 weeks	Flavonoid compounds contained in *M. oleifera* leaf extract reduce blood glucose levels through their antioxidant activity	Sierraacampos *et al.*, 2020
Methanolic extract	Albino rats weighing 120–180 g and obtained from Madonna University, Elele, Nigeria	*In vivo*: Alloxan-induce rats, doses of 130 mg/kg BW, sample doses of 100, 200, and 400 mg/kg BW in 4 weeks	Significantly reduced blood glucose levels. Results of groups 3, 4, and 5 (172.0±4.75, 142.9±47.25, 70.6±24.46 mg/dL, respectively) where indicated by a decrease ($P<0.05$) in induced rat blood glucose levels when compared with group 2 (316±47.17 mg/dL), which was only exposed to alloxan	Udeogu *et al.*, 2019
Aqueous extract	Adult normoglycemic male albino rat (Sprague-Dawley) weighing 180–200 g, 12 months old	Streptozotocin-induced rats, doses of 60 mg/kg BW, sample doses of 200 mg/kg in 21 days	It reduced fasting blood sugar and blood sugar levels after eating up to 69% and 51%, respectively, comparable to glipizide as the positive control	Yassa and Tohamy, 2014
Aqueous extract	Male Wistar rat	Streptozotocin-induced rats, doses of 45 mg/kg BW, sample doses of 100 mg/kg BW in 3 weeks	It lowers blood glucose levels, stimulates insulin production, and inhibits α-amylase and α-glucosidase enzyme activity	Khan *et al.*, 2017
Methanolic extract	Male Wistar rats weighing 150–180 g	*In vivo*: Alloxan-induced rats, doses of 120 mg/kg BW, sample doses of 300 and 600 mg/kg BW in 6 weeks	It significantly reduced fasting blood sugar levels and increased insulin secretion	Olayaki *et al.*, 2015
Aqueous extract	Female Wistar rats weighing 130±10 g, 90 days old, obtained from National Research Center Animal House, Giza, Egypt	*In vivo*: Balb/c mice, the sample was administered orally at 600 mg/kg BW in obese female rats every day for 12 weeks	It reduces blood sugar levels	Metwally *et al.*, 2017
Ethanol 50% extract	Male Sprague-Dawley rats, 6 weeks old	*In vivo*: Sprague-Dawley rats, given a high-fat, high-fructose diet for 60 days	It significantly reduced fasting blood sugar levels. *M. oleifera* treatment for 30 days significantly mitigates features of metabolic syndrome	Irfan *et al.*, 2020
Ethanol 80% extract	Male rats C57BL6	*In vivo*: Mice, 200 mg/kg for 7 days	It considerably lowered blood glucose levels. The leaf extract, 400 mg/kg, treatment returned insulin levels to average values ($P<0.05$ compared with the diabetic control group)	Attakpa *et al.*, 2017
Flavonoid kaempferol	Mice	Streptozotocin-induced mice, doses of 200 mg/kg/BW, 1 week, sample doses of 50 mg/kg/day	It considerably lowers blood sugar levels after eating (postprandial), but it takes 2–4 weeks to lower fasting blood sugar levels	Alkhalidy *et al.*, 2018

DM: Diabetes mellitus, *M. oleifera: Moringa oleifera*, BW: Body weight