

Full Length Research Paper

Evaluation of antihyperglycemic, antiradical and acute oral toxicity activities of aqueous leaves extract of *Moringa oleifera* Lam (*Moringaceae*) from Benin in normal rats

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Received 7 November, 2015; Accepted 4 May, 2017

Moringa oleifera is a commonly cultivated plant in the world. It has a high economic impact owing to its medicinal and nutritional values. Leaves of *M. oleifera* also contain various constituents that are useful for therapeutic purposes. The aim of this study is (i) to perform phytochemical screening, and to check antiradical and antihyperglycaemic activities of *M. oleifera* aqueous leaves extract (ii) to control the impact of this aqueous extract on the liver parameters of wistar rats. The analysis was carried out on a few large families of bioactive molecules such as triterpenoids, alkaloids, anthraquinones, coumarins, flavonoids, saponins, tannins, anthocyanes, leucoanthocyanes, reducing sugar, mucilage and cyanogenic. With a concentration of 2.47 ± 0.26 mmol EAA/mg, the plant has a good antioxidant activity. Antihyperglycemic capacity was evaluated by using oral glucose tolerance test. The α, α -diphenyl- β -picrylhydrazyl (DPPH) method was used to show antiradical activity. The impact of aqueous leaves extract of *M. oleifera* on some biochemical parameters of Wistar rats (150 to 200 g) was shown. The contents of total phenolic, flavonoids and condensed tannins were respectively 46.242 ± 2.44 (mmolEAA/mg of extract); 10.80 ± 0.64 (mg Eq of rutine/mg of extract); 2.85 ± 1.018 (mg Eq of catechine/mg of extract). The results of the acute oral toxicity test showed that the dose of 2000 mg/kg was non-toxic to the rats. The haematological and blood parameters did not change after administration of the plant aqueous extract. Glycemia reduction was observed with the dose of 500 mg/kg. The important results obtained in this study explain the abundant use of this plant by the populations in the treatment of diabetes. However, it would be necessary to confirm the non-toxicity of the plant by histological studies of the liver and kidneys.

Key words: *Moringa oleifera*, aqueous leaves extract, antihyperglycemic activity, acute oral toxicity, *in vivo*.

INTRODUCTION

Diabetes mellitus is a major degenerative disease in the world today (Ogbonnia et al., 2008). The fundamental defect in diabetes mellitus is an absolute or relative lack of biologically active insulin, which results in the impairment of uptake and storage of glucose, and reduced glucose utilization for energy purpose (Tiwari and Rao, 2002). According to World Health Organization projection, the prevalence of diabetes is likely to increase by 35% (Sangeeta et al., 2010). Currently, there are over 150 million diabetics world-wide and this is likely to increase to 300 million or more by the year 2025. The prevalence of diabetes mellitus in Benin is 2.9% (Djrolo et al., 2011).

Although, anti-diabetic drugs are accessible, in many cases, the treatment is not suitable because these drugs are not able to heal this infection and present side effects. In the same way, the high cost of the anti-diabetic drugs directs the populations of low and middle-income countries to resort to the medicinal plants. Medicinal plants constitute an important source of potential therapeutic agents for diabetes and hyperlipidemia (Adisakwattana et al., 2011). Most of the plants prescribed for diabetes mellitus are not edible and therefore, the studies on edible plants which have an antihypoglycemic effect would be of great value in the dietary management of the disease. *M. oleifera* is used in traditional medicine all over the world to cure many diseases (India, Pakistan, ancient Rome, Greece and Egypt).

Different parts of this plant such as the leaves, roots, seed, bark and flowers were used in indigenous medicine for the treatment of some pathologies. Leaves were known to act as cardiac and circulatory stimulants, possess antitumor, antipyretic, antiepileptic, anti-inflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, cholesterol lowering, antioxidant, antidiabetic, hepatoprotective, antibacterial and antifungal activities, and are being used for the treatment of different ailments in the indigenous system of medicine, particularly in South Asia (Farooq et al., 2007). Fresh root is acrid and vesicant (has the taste of horse-radish). Internally, it is used as stimulant, diuretic and antilithic. Seeds are acrid and stimulant. Bark is emmenagogue and even abortifacient, antifungal and antibacterial. Flowers are cholagogue, stimulant, tonic and diuretic, and useful to increase the flow of bile. The plant is also a cardiac circulatory tonic and antiseptic.

Ethnobotanical studies along different phytodistricts in Benin showed the use of *M. oleifera* as one of the plants involved in the traditional medicine in the treatment of

diabetes pathology (Laleye et al., 2015). As in most cases, a lot of plants are used in indigenous medicine to treat diseases, it is very difficult to say either if the results are from action of a single plant or a synergy of all plants. To elucidate this aspect, there is a need to evaluate the action of this plant extract in order to provide scientific proof and its role in the treatment of pathologies. In the same way, the phytochemical screening of aqueous extract of *M. oleifera* was done to evaluate its action on diabetes.

MATERIALS AND METHODS

Plant material

The leaves of *M. oleifera* were collected from Abomey-Calavi (Benin) city during the month of January to February, and were certified at the National Herbarium of Benin. A voucher specimen (AA6533/HNB) was reserved in this herbarium. The plant material was air dried at room temperature in the laboratory and further dried in the oven for 1 week at a temperature of 24°C. The leaves were then powdered using mortar and pestle.

Preparation of the water extract

The powder was hot macerated with distilled water and then the extract obtained was filtered, concentrated by rotary vacuum pump to get the solid mass because the main methods of *M. oleifera* remedy preparations in Benin is the maceration.

Animal treatments

In this study, albino Wistar rats (male and female) weighing 150 to 200 g and 4 months old were used. Animals were maintained under standard conditions and guarded in iron cages with easy access to water and food. The temperature of the room was maintained at 25°C and the relative humidity is between 35 and 60%. Animals were submitted to an alternated cycle of light (12 h) and dark (12h).

Drugs and chemicals

Different drugs and chemicals were obtained from Sigma (USA), Aldrich (Milwaukee, USA): sodium carbonate (Na₂CO₃), gallic acid, methanol, hydrochloric acid (HCl), Folin-Ciocalteu reagent, sodium nitrite (NaNO₂), vanillin, aluminum chloride (AlCl₃), sodium hydroxide (NaOH), 2,2-diphenyl-1-picrylhydrazyl, ferric chloride (FeCl₃), ammoniac, sulfuric acid, acetic anhydride, chloroform, toluene, dinitrobenzoic acid, phosphomolybdenum, catechin and potassium ferricyanide

Phytochemical screening

The freshly prepared crude leaves extract of *M. oleifera* was subjected to qualitative phytochemical analysis for the presence of

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various classes of active chemical constituents such as tannins, saponins, glycosides, flavonoids, alkaloids using the methods described by Houghton and Raman (1998).

Quantification of the bioactive molecules

Determination of total phenolic content

Singleton et al. (1999) was used to determine total phenolic content. 125 μ l of aqueous extract at the concentration of 1 to 5 mg/ml was diluted into 250 μ l of distilled water. Then, 625 μ l of Folin-Ciocalteu reagent was added and gently mixed. After 2 min of homogenization, 500 μ l of 20% sodium carbonate was added. The contents were mixed and allowed to stand for 2 h. The optical density of the blue colored samples was measured at 760 nm. Gallic acid was used as standard. The total phenolic content was expressed in mg/g of gallic acid equivalent of mixture.

Determination of total flavonoid content

Total flavonoid content of the extract was measured based on method described by Kim et al. (2003) with slight modifications. According to this method, 400 μ l of extract was mixed with 500 μ l of distilled water and then 120 μ l of 5% sodium nitrite was added to the mixture and allowed to stand for 5 min, followed by the addition of 120 μ l of 10% of AlCl_3 to the mixture which was mixed using a vortex. 800 μ l of 1 M NaOH was added after 6 min and the mixture was incubated for 15 min in darkness. Rutin solution was used as reference and the content in flavanoids was expressed as mg of rutin equivalent/1 mg of sample. The calibration curve established with the reference solution was used to read directly the concentration values.

Determination of condensed tannins content

Broadhurst and Jones (1978) method modified by Heimler et al. (2006) was used to appreciate condensed tannins content. 1.5 ml of vanillin solution initially dissolved in methanol for a final concentration at 4% was mixed with 500 μ l of extract, 2 ml of methanol and 1.5 ml of concentrated hydrochloric acid. The mixture was incubated for 15 min and the absorbance was taken at 500 nm. Catechin solution was used as reference and the content of condensed tannins was expressed as mg of catechin equivalent/mg of extract.

Total antioxidant capacity assay (TAO)

The ability of the plant extract to trap free radicals explains their antioxidant capacity. The method described by Lamien-Meda et al. (2008) was used. 0.75 ml of different concentrations (from 0.5 to 3.5 mg/ml) of leaves extract were mixed with 1.5 ml of DPPH solution (0.4 mg/ml in methanol). Then, the mixture was incubated at room temperature for 15 min in a dry bath room. Ascorbic acid solution is used as standard and the total antioxidant activity was expressed as millimol of ascorbic acid equivalent per gram of aqueous extract.

Acute toxicity studies

This assay was done in accordance with Organization for Economic Cooperation and Development (OECD, 2001) guideline. Three female and non-gravid Wistar rats weighing 150 to 200 g received by gavage, 2000 mg/kg aqueous extract of *M. oleifera* after being

kept on fasting the previous night. The animals are observed closely during the first four hours and daily for 14 days (for weight change, tremors, convulsion, salivation, diarrhea, lethargy, sleep, coma, and death, changes in the skin, fur, eyes and behavioral pattern). At the end of 14 days, a histological study was performed on some organs (liver and kidney) and biochemical parameters were measured.

Collection of blood and serum samples

Anesthetized rats underwent cervical decapitation. The blood was collected in heparinized bottles for hematological studies. Blood samples taken from non-heparinized bottles were allowed to coagulate. The serum was separated from the clot and centrifuged in clean bottles for biochemical analysis.

Hematological measurements

The hematological examinations were done using blood samples collected from retro-orbital of the experimental rats and conserved in capillary tubes (EDTA). These examinations using Toro and Ackermann (1975) and Duncan et al. (1994) included red blood cell counts, white blood cells and platelets hemoglobin, hematocrit, mean globular volume (VGM), average corpuscular content in hemoglobin (TCMH), the mean corpuscular concentration in hemoglobin (CCMH), aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Creatinine levels and serum urea were measured using the method described by Coles (1986).

Anti-hyperglycemic activity of *M. oleifera*

Pareek et al. (2009) method was used to evaluate anti-hyperglycemic activity by oral glucose tolerance test (OGTT). 30 rats divided into 6 lots of five rats were constituted before the experiment (OGTT), all the rats were beforehand submitted to a non-hydric fast for 16 h. 500 and 1000 mg/kg of aqueous extract were administered to the lots 3 and 4. Lots 1 and 2 received each, a dose of 5 ml/kg of Glibenclamide. The control received distilled water with a dose of 10 ml/kg. D-glucose was administered to the rats with a dose of 3 g/kg after half an hour. SD CHECK glucometer was used to take the glycemia in a sequential way every 30 min from start point corresponding to T0 up to 120 min corresponding to T120. The final measure was taken at T 180 min.

Statistical analysis

Statistical analysis of the data was done and expressed as a mean using Minitab Version 1.0 software. The differences obtained from the univariate variance analysis (ANOVA) were reported using the Kruskal-Wallis test. Correlations between different values were expressed graphically using Graph Pad Prism version 5. For $p \leq 0.05$, the results are statistically significant.

RESULTS AND DISCUSSION

Phytochemical screening and bioactive molecules quantification

Only three families of compounds were absent from the ten important families of compounds sought in the extract (Table 1). They are leucoanthocyan, coumarin and cyanogenic glycoside. The content of phenolic compounds,

Table 1. Phytochemical analysis of *M. oleifera* leaves extract.

Chemical compounds class	Test	Test result
Alkaloids	General test: Dragendorff reagent	+
Catechin tannins	Stiasny reagent	+
Gallic tannins	Saturation of Na acetate + a few drops of FeCl ₃ , 1%	+
Flavonoids	Shinoda reagent (cyanidine reaction)	+
Anthocyanes	Adding some drops of HCl 5% to 1 ml of decocted + alalinisation (with drops of ammoniac 50%)	+
Leucoanthocyanes	Shinoda reagent (chlorhydric alcohol)	-
Quinonic derivatives	Born- Trager reaction: concentrated HCl, diluted HCl	-
Saponosides	Foam index (FI) of diluted aqueous decoction (positive if IF ≥ 100, measuring foam height ≥ 1 cm)	+
Reducing sugars	Fehling's test	+
Triterpenoids	Liebermann-Buchard reaction (acetic anhydride-sulfuric acid 50: 1) Kedde reaction (dinitrobenzoic acid 2% in ethanol + NaOH (1 N) 1:1)	+
Cyanogenic derivatives	Ammoniac at 25%: high fluorescence	-
Mucilages	Chloroform + ammoniac: red ± highly coloration	+
Coumarins	Extraction, elution with toluen + AcEt (97/3), revelation with sulfuric anysaldehyde or vanilin	-

+ Present, - absent.

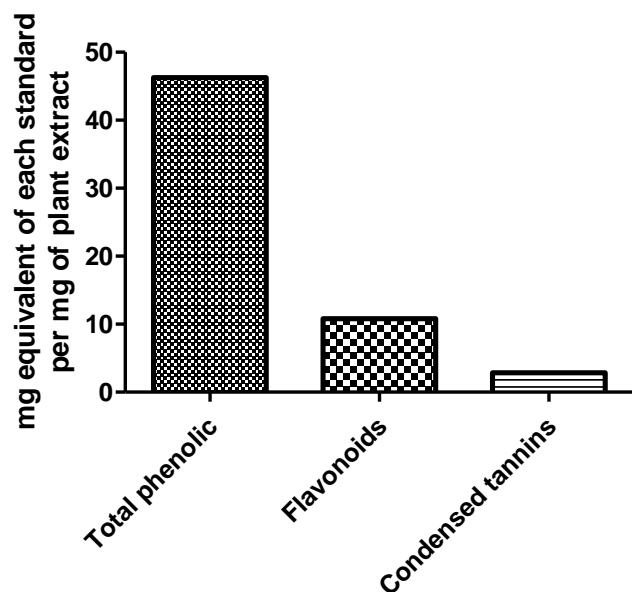


Figure 1. Total phenolic, flavonoids and condensed tannins content of *M. oleifera* leaf extract.

flavonoids and condensed tannins respectively in the aqueous leaves extract of *M. oleifera* are 46.242±2.44, 10.80±0.64 and 2.85±1.018 (Figure 1).

Total antioxidant capacity assay (TAO)

The antioxidant capacity of the plant leaves extract was

2.47 ± 0.26 mmol equivalents of ascorbic acid per mg of leaves extract. Total antioxidant capacity represents both oil soluble and water soluble antioxidants that are capable of scavenging reactive oxygen species and protects from chronic diseases such as cancer, diabetics and arthritics (Mrudula et al., 2014).

Acute toxicity studies

It was observed that no rat that received the aqueous extract of the plant at a dose of 2000 mg/kg died. This result shows that the concentration administered to the rats is below the toxicity threshold, 2000 mg/kg which is therefore lower than the LD50. It was observed that the rats used in this test gained weight as those of the control group. This is contrary to the effects of toxicity. Figure 2 presents the evolution of animal's weight during the 14 days.

Determination of hematological and biochemical parameters

Table 2 presents the results obtained from different measurements of hematological parameters. No differences were observed between control animals and experimental animals. These results showed that the aqueous extract of *M. oleifera* leaves has no impact on the metabolic parameters of rats.

Table 3 presents evolution of some biochemical parameters in comparison with control rats. Maintaining of metabolic equilibrium requires that these parameters

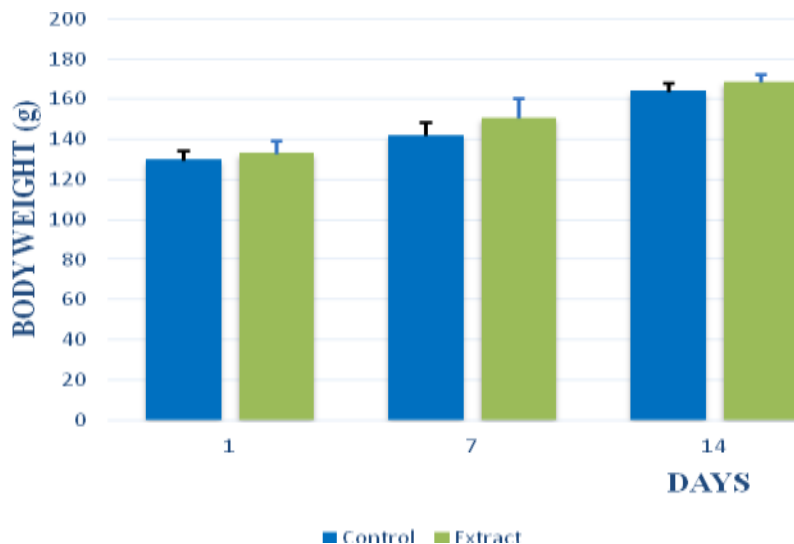


Figure 2. Effects of *M. oleifera* on the body weights of rats.

Table 2. Hematological parameters in rats after administering *M. oleifera* extract.

Parameter	RBCs (T/L)	PLT (G/L)	WBCs (T/L)	Hb (g/dl)	HTE (%)	MCV (fL)	MCHC (%)	MCH (pg)
Control rat	5.22±0.10	821.30±29.6	3.17±0.57	11.4±0.2	32±1.0	62.33±2.52	27.93±0.40	21.80 ±0.40
Experimental rats	5.26±0.22	831.30±4.60	3.50±0.78	11.60±0.65	32.67±3.51	62.33±2.52	28.10±1.45	22.03±0.80
P value	0.805	0.753	0.592	0.664	0.782	0.773	0.866	0.696

Hb: Hemoglobin; MCV: mean cell volume; MCHC: mean cell hemoglobin concentration; RBCs: red blood cells count; WBCs: white blood cells count; PLT: platelets; HTE: hematocrit; MCH: mean corpuscular hemoglobin; Hb: hemoglobin.

Table 3. Determination of biochemical parameters of rats after treatment with *M. oleifera* extract.

Parameter	Creatinine (mg/L)	Urea (g/L)	ALT (UI/L)	AST (UI/L)
Control	7.47±1.10	0.30±0.01	88.67±4.16	144.67±7.57
Aqueous extract	7.67±0.42	0.31±0.04	79±10.5	152.0±30
P-value	0.796	0.826	0.277	0.738

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

are constant. As compared to the 2000 mg/kg dose, no significant differences were observed in these parameters, as the $p > 0.005$. For both hematological and biochemical parameters, no significant difference ($p > 0.005$) was observed.

Anti-hyperglycemic activity of *M. oleifera*

From the analysis of the graphs presented in Figure 3, distilled water showed a high level of hyperglycemic activity from T0 to T90, after which a low decrease up to T180 was noticed. For plant leaves extract, the two

concentrations (500 and 1000 mg/kg) presented the same effect on the hyperglycemic activity. An increase of the hyperglycemic level was observed only for the first half of time. Then, from T30, hyperglycemic activity decreased progressively until T180 for both concentrations.

DISCUSSION

Traditional medicine is the oldest medicine used for the treatment of many diseases and by rural as well as urban people. This medicine was based mostly on the use of

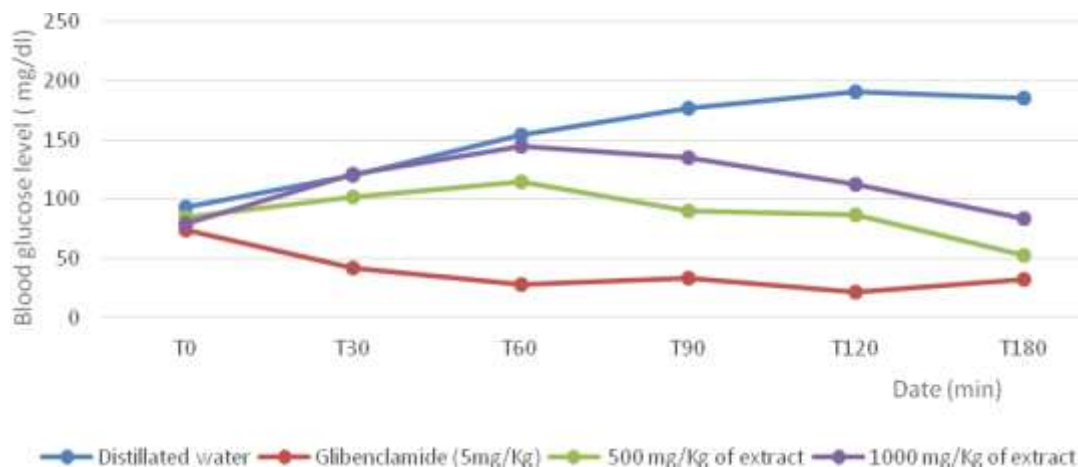


Figure 3. Effect of *M. oleifera* leaves extract on blood glucose level in rats. T0, T30, T60, T90, T120 and T180 stands for time interval of hyperglycemic activity in minutes.

diverse plants which revealed different pharmacological properties. These properties were attributed to the presence of some secondary plants metabolites. Indeed, the phytochemical screening carried out on the aqueous extract of *M. oleifera* leaves revealed the presence of phenolic compounds, sugar and other substances. Their presence confirms in certain proportions, their efficiency in the treatment of many diseases. Most medicinal plants are the important source of bioactive molecules that contribute to the reduction of risk and progression of certain acute and chronic diseases such as heart diseases, cancer, diabetes, etc. (Ali et al., 2008). Farooq et al. (2007) also revealed the presence of alkaloids, tannins and flavonoids in *M. oleifera* leaves extract but in different proportions (Teteh et al., 2013). The age of the plant, the harvest period of the plant, the climate, the method of extraction, the type of soil could explain the differences noticed. In this case, phenolic compounds content was higher than the remainder and indicated that it was the most important compound in *M. oleifera* leaves extract. This result could probably justify the antioxidant activity of the leaves. Phenolic compounds were reported to have multiple biological effects, including antioxidant activity (Jaehak et al., 2014). The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Singleton et al., 1965).

Regarding antihyperglycemic activity, it was shown that leaves extract presented this activity. A maximum action on blood glucose level decrease was obtained with a dose of 500 mg/kg. The findings are in agreement with the result of Edoga et al. (2013) who showed that the leaves extract of *M. oleifera* reduced blood sugar levels in the normoglycemic rats, this effect was dose dependent. The antihyperglycemic effect of the plant may be due to

the presence of alkaloids, flavonoids, steroids and other constituents present in the leaves extract which could act synergistically or independently in lowering the blood sugar level. Some alkaloids are known hypoglycemic agents. For instance berberine, a quaternary ammonium salt from the protoberberine group of isoquinoline alkaloids has been used successfully in experimental models of diabetes mellitus, berberine was shown to possess insulin sensitizing effect (Wang et al., 2010). Also, flavonoids, being polyphenolics, are known to be hypoglycemic. The antidiabetic activity of four flavonoids-boswellic acid, ellagic acid, quercetin and rutin were demonstrated in rats and the proposed mechanism of action was by increasing the peripheral utilization of glucose and inhibiting the glucose transporter activity from intestine (Jadhav and Puchchakayala, 2012). Thus, *M. oleifera* might decrease the velocity of gastric emptying, a major determinant of postprandial glycemia (Horowitz et al., 2002). Some herbal medicines were reported to inhibit gastric emptying and also glucose uptake in the small intestine (Matsuda et al., 1998). High fiber diets decrease postprandial blood glucose by slowing the rate of food passage from the stomach to the small intestine (Tsai and Peng, 1981).

Administration of *M. oleifera* leaves extract did not affect blood biochemical parameters. In the same way, the toxicity test on Wistar rats revealed a gain of body weight with all groups of rats even when the dose was 2000 mg/kg. The increase in the body weight of rats might be due to the fact that *M. oleifera* is rich in amino acids, vitamins and minerals, particularly iron (Booth and Wickens, 1988; Mori et al., 2009). The results obtained show that the LD50 is greater than 2000 mg/kg. This result is in concordance with the work of Ojo et al. (2013) who used the dose of 5000 mg/kg. At doses above this level; however, the animals may exhibit some toxic changes. The plant is safe for consumption and for

medicinal uses.

Conclusion

The result obtained from this research study revealed that aqueous leaves extract of *M. oleifera* exhibited diverse bioactive compounds with different properties. The plant leaves extract presented no acute toxicity and could be safe for consumption as traditional vegetable and for medicinal uses. With its contribution in the decrease of blood glucose level, this result confirmed its use by traditional therapist for treatment of diabetes. However, it is important that future study should contribute to identifying and characterizing the crude extract specific molecules involved in the treatment of each type of the disease.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

- Adisakwattana S, Chanathong B (2011). α -glucosidase inhibitory activity and lipid-lowering mechanisms of *Moringa oleifera* leaf extract. *Euro. Rev. Med. Pharmacol. Sci.* 15:803-808.
- Ali SS, Kasoju N, Luthra A, Singh A, Sharanabasava H, Sahu A, Bora U (2008). Indian medicinal herbs as sources of antioxidants. *Food Res. Int.* 41(1):1-15.
- Booth FE, Wickens GE (1988). Non-timber uses of selected arid zone trees and shrubs in Africa. *FAO conservation guide*. Pp. 92-101.
- Broadhurst RB, Jones WT (1978). Analysis of condensed tannins using acidified vanillin. *J. Sci. Food Agric.* 29:788-794.
- Coles (1986). *Veterinary Clinical Pathology*. W.B. Saunders' Co. Philadelphia. pp. 5-87.
- Djirolo F, Gbary A, Houinato D, Fambo D, Djigbénoudé O (2011). Prevalence of diabetes mellitus and associated factors in the general population in Benin. *World Diabetes Congress. Abstract Books*.
- Duncan JR, Prasse KW, Mahaffey EA (1994). *Veterinary Laboratory Medicine Clinical Pathology*. Iowa State University Press: Ames, pp. 94-96.
- Edoga CO, Njoku OO, Amadi EN, Okeke JJ (2013). Blood Sugar Lowering Effect of *Moringa Oleifera* Lam in Albino Rats. *Int. J. Sci. Technol.* 3 (1):88-90.
- Farooq A, Sajid L, Muhammad A, Anwarul-Hassan G (2007). *Moringa oleifera*: a food plant with multiple medicinal uses. *Phytother. Res.* 21:17-25.
- Heimler D, Vignolini P, Dini MG, Vincieri FF, Romani A (2006). Antiradical activity and polyphenol composition of local Brassicaceae edible varieties. *Food Chem.* 99(3):464-469.
- Horowitz M, O'Donovan D, Jones KL, Feinle C, Rayner CK, Samsom M (2002). Gastric emptying in diabetes: clinical significance and treatment. *Diabet. Med.* 19:177-194.
- Houghton PJ, Raman A (1998). *Laboratory Handbook for Fractionation of Natural Extracts*. Chapman and Hall, London, P 199.
- Jadhav R, Puchchakayala G (2012). Hypoglycemic and antidiabetic activity of flavonoids: boswellic acid, ellagic acid, quercetin, rutin on streptozotocin nicotinamide induced type 2 diabetic rats. *Int. J. Pharm. Sci.* 4(2):251-256.
- Jaehak L, Kandhasamy S, Mihae K, Kim J, Kim D, Kim S, Kim GY, Kim S, Jhoo JW (2014). Antioxidant, inhibition of α -glucosidase and suppression of nitric oxide production in LPS-induced murine macrophages by different fractions of *Actinidia arguta* stem. *Saudi J. Biol. Sci.* 21(6):532-538.
- Kim DO, Chun OK, Kim YJ, Moon HY, Lee CY (2003). Quantification of polyphenolics and their antioxidant capacity in fresh plums. *J. Agric. Food Chem.* 51(22):6509-6515.
- Laleye FOA, Mensah S, Assogbadjo AE, Ahissou H (2015). Diversity, Knowledge, and use of Plants in traditional treatment of diabetes in the Republic of Benin. *Ethnobotany Res. Appl.* 14:231-257.
- Lamien-Meda A, Lamien CE, Compaoré MMH, Meda NTR, Kiendrebeogo M, Zeba B, Millogo JF, Nacoulma OG (2008). Polyphenol content and antioxidant activity of fourteen wild edible fruits from Burkina Faso. *Molecules* 13(3):581-594.
- Matsuda H, Murakami T, Li Y, Yamahara J, Yoshikawa M (1998). Mode of action of escins Ia and IIa and E,ZSenegin II on glucose absorption in gastrointestinal tract. *Bioorg. Med. Chem.* 6:1019-1023.
- Mori S, Cameldi I, Pardini A (2009). *Moringa oleifera*: a promising multipurpose tree for tropical and subtropical areas. *Associazione Scienze Agrarie Tropicali* 1:1-2.
- Mrudula CM, Ashish AP, RituRaval (2014). Phytochemical quantification and antioxidant capabilities of *Moringa olifera*, *Basella alba* and *Centella asiatica* leaf source. *Int. J. Innov. Res. Sci. Eng. Technol.* 3(2): 9243-9251.
- OECD (2001) Guideline for testing of chemicals. Guideline 420: Acute Oral Toxicity-Fixed Dose Procedure. 15:254-365.
- Ogbonnia SO, Odimegwu JI, Enwuru VN (2008). Evaluation of hypoglycemic and hypolipidemic effects of ethanolic extracts of *Treculia africana* Decne and *Bryophyllum pinnatum* Lam and their mixture on streptozotocin (STZ) - induced diabetic rats. *Afr. J. Biotechnol.* 7(15):2535-2539.
- Ojo NA, Igwenagu E, Badau SJ, Sambo N, Ngulde SI, Adawaren EO, Madziga HA, Yahi D, Mbaya YP, Bargu JS, Simon J, Ndahi JJ, Auwal MS, Dibila HM (2013). Acute Toxicity and Effect of Aqueous Extract of *Moringa oleifera* leaves on organs and tissues in rats. *J. Environ. Issues Agric. Dev. Ctries.* 5(2):41-50.
- Pareek H, Sharma S, Khajja BS, Jain K, Jain GC (2009). Evaluation of Hypoglycemic and Anti-Hyperglycemic Potential of *Tridax procumbens* (Linn). *BMC Complement. Altern. Med.* 9(1):48.
- Sangeeta C, Rahul J, Saxena RC, Chaurasia ID, Rajeev S (2010). Hyperglycemic activity of *Eugenia jambolane* in Streptozotocin induced diabetic rats. *J. Chem. Pharm. Res.* 2(5):458-460.
- Singleton VL and Rossi JA (1965). Colorimetry of total phenolics with phosphomolybdicphosphotungstic acid reagent. *Am. J. Enol. Vitic.* 16(3): 144-158.
- Singleton VL, Orthofer R, Lamuela-Raventos RM (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol.* 299:152-178.
- Teteh A, Lawson E, Tona K, Decuypere E, Gbeassor M (2013). *Moringa oleifera* leaves: hydro-alcoholic extract and effects on growth performance of broilers. *Int. J. Poult. Sci.* 12(7):401-405.
- Tiwari AK, Rao JM (2002). Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. *Curr. Sci.* 83:30-38.
- Toro G, Ackermann PG (1975). *Practical Clinical Chemistry*. 1st ed. Little Brown and Co. Inc., Boston, USA.
- Tsai AC, Peng B (1981). Effects of locust bean gum on glucose tolerance, sugar digestion, and gastric motility in rats. *J. Nutr.* 111:2152-2156.
- Wang Y, Campbell T, Perry B, Beaurepaire C, Qin L (2010). Hypoglycemic and insulin sensitizing effects of berberine in high-fat diet- and streptozotocin-induced diabetic rats. *Metab. Clin. Exp.* 60(2):298-305.