



In vitro inhibitory potentials of ethanolic extract of *Moringa oleifera* flower against enzymes activities linked to diabetes

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ARTICLE INFO

Article Type:
Original Article

Article History:
Received: 14 November 2020
Accepted: 29 April 2021

Keywords:
Moringa oleifera
Antioxidant activity
 α -Amylase
 α -Glucosidase
Type II diabetes

ABSTRACT

Introduction: Diabetes mellitus (DM) has been recognized as the seventh leading cause of global mortality; however, researchers seek alternative means to manage the menace. The current study sought to investigate antioxidant potentials, α -amylase and α -glucosidase inhibitory activities of ethanolic extract of *Moringa oleifera* flower *in vitro*.

Methods: Antioxidant properties of the extract were appraised by assessing its inhibition against 1,1-diphenyl-2-picrylhydrazyl (DPPH), hydroxyl (OH[•]) and hydrogen peroxide (H₂O₂) free radicals, as well as ferric reducing antioxidant power (FRAP), the antidiabetic activity was evaluated by α -amylase and α -glucosidase inhibition.

Results: In this study, ethanolic extract of *M. oleifera* flower demonstrated a significant ($P < 0.05$) inhibition against DPPH free radical (43.57–83.56%) in a concentration-dependent manner, while FRAP (101.76 ± 1.63 mg/100 g), OH[•] scavenging ability (71.62 ± 0.95 mg/100 g), and H₂O₂ free radical scavenging capacity (15.33 ± 1.20 mg/100 g) were also observed. In the same manner, ethanolic extract of *M. oleifera* flower revealed a significant ($P < 0.05$) inhibition against α -amylase (IC₅₀ = 37.63 mg/mL) and α -glucosidase activities (IC₅₀ = 38.30 mg/mL) in the presence of their respective substrates in a concentration-dependent manner in comparison with acarbose.

Conclusion: Ethanolic extract of *M. oleifera* flower could be useful as an alternative phytotherapy in the management of DM, having shown a strong antioxidative capacity and substantial inhibition against the activities of key enzymes involved in carbohydrate hydrolysis *in vitro*.

Implication for health policy/practice/research/medical education:

Ethanolic extract of *Moringa oleifera* flower possesses significant antioxidant and antidiabetic potentials and might be used as an alternative phytotherapy in the management of diabetes mellitus.

Please cite this paper as: Olasehinde OR, Afolabi OB, Omiyale BO, Olaoye OA. *In vitro* inhibitory potentials of ethanolic extract of *Moringa oleifera* flower against enzymes activities linked to diabetes. J Herbmed Pharmacol. 2021;10(4):408-414. doi: 10.34172/jhp.2021.48.

Introduction

Diabetes mellitus (DM) is a complex multi-systemic disorder characterized by a relative or absolute impaired insulin secretion or its actions leading to disturbances in carbohydrate, protein, and lipid metabolisms (1). This metabolic disorder has been reported to affect approximately 4% of the global population worldwide with a projection of 5.4% rise by 2025 (2). However, two major types of DM have been widely recognized, i.e., insulin-dependent (IDDM, type 1) and non-insulin-dependent diabetes mellitus (NIDDM, type 2). IDDM is an autoimmune disease characterized by a local

inflammatory reaction in and around islets followed by selective destruction of insulin-secreting cells, whereas NIDDM is characterized by peripheral insulin resistance and impaired insulin secretion (3).

Several modifications have been reported in DM as a result of changes in body proteins that in turn lead to complications in the eyes (retinopathy), kidneys (nephropathy), nerves (neuropathy), and arteries (atherosclerotic vascular disease) (4). Although, various oral hypoglycemic drugs exist alongside insulin therapy, however, there is no promising therapy to cure DM (5). The conventional management of DM includes reduction

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of demand for insulin, stimulation of endogenous insulin secretion, and inhibition of degradation of oligo- and disaccharides (5,6). Carbohydrate-metabolizing enzymes such as α -glucosidases and α -amylases have been reported to be responsible for the breakdown of oligo- and/or disaccharides to monosaccharides. However, inhibition of these enzymes has been reported to play a significant role in the management of NIDDM (7,8). The activities of potential inhibitors of carbohydrate-metabolizing enzymes such as acarbose have been well elucidated and reported (9). Synthetic hypoglycemic agents have been reported for their limitations such as non-specificity, serious side effects (e.g., bloating, abdominal discomfort, diarrhea, and flatulence) (10), and eventual failure in alleviating DM complications (9).

Phytotherapy has recently been reported to play promising roles in the management of DM (9). *Moringa oleifera* is a tropical and perennial softwood tree with timber of low quality, which for centuries parts of the plant (roots, leaves, stem, and seeds) are edible and have been advocated for nutritional purposes and traditional medicinal and industrial uses (11). Various parts of *M. oleifera* have been reported for their several biological activities. Report shows that its leaves have purgative, antimicrobial, and hypoglycemic properties (12). Its stem bark has been proven to possess anti-cancer, anti-ulcerative, and anti-inflammatory properties (12). Different parts of this plant contain a profile of important minerals and are a good source of protein, vitamins, β -carotene, amino acids, and various phenolic compounds (13). Hence, this study was designed to appraise the antidiabetic activities of the ethanolic extract of *M. oleifera* flowers through the inhibition of α -glucosidase and α -amylase enzymatic activities as well as the antioxidant properties.

Materials and methods

Chemicals used

Ethanol was procured from Merck (Darmstadt, Germany), 1,1-diphenyl-2-picrylhydrazyl (DPPH), acarbose, α -amylase (porcine, EC 3.2.1.1) and α -glucosidase (*Saccharomyces cerevisiae*; EC 3.2.1.20) were purchased from Sigma Chemical Co. (Sigma-Aldrich, Sternheim, Germany). Butylated hydroxytoluene (BHT) and gallic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and reagents were of analytical grade and prepared in all-glass apparatus.

Sample collection and preparation

Sample collection

M. oleifera flowers were obtained from Ekute quarters, Ado-Ekiti, Ekiti State, Nigeria. A voucher sample was deposited for authentication at the herbarium unit, Department of Plant Biology, University of Ilorin, Kwara State. A voucher number (U.I.H 729) was provided for it from the data base.

Preparation of ethanolic extract of *M. oleifera* flower

Fresh flowers of *M. oleifera* were air-dried at room temperature (37°C) for fourteen (14) days. The dried flowers (50 g) were then pulverized using an automated blender, after which the resulting powder was extracted in 500 mL 95% ethanol for 72 hours at room temperature with a constant agitation. The extract was then filtered using Whatman No. 1 filter paper, and the filtrate was concentrated to dryness in a water bath at 40°C.

In vitro antioxidant activity assays

Ferric reducing antioxidant power (FRAP) assay

Ferric reducing antioxidant potential of the extract was carried out using the method of Pulido et al (14). Extract (2.5 mL) was mixed with 2.5 mL 200 mM phosphate buffer (pH 6.6) and 2.5 mL 1% potassium ferricyanide. Solution was incubated for 20 minutes at 50°C in a water bath and 2.5 mL 10% trichloroacetic acid (TCA) was added. The resulting solution was then centrifuged for 10 minutes at 3000 rpm. Then, 5 mL of the filtrate was mixed with an equal volume of distilled water and 1 mL 0.1% FeCl₃. The mixture was read at 700 nm, and gallic acid was used as standard.

DPPH free radical scavenging activity

DPPH free radical scavenging ability of the extract was carried out by Gyamfi et al (15). Diluted extract (1 mL) was mixed with 1 mL 0.4 mM DPPH solution in methanol. The mixture was incubated in the dark for 30 minutes and was read at 516 nm. DPPH radical scavenging assay was expressed as percentage (%) control inhibition. Butylated hydroxytoluene (BHT) was used as a standard.

$$\text{DPPH inhibition (\%)} = \frac{A_{540}^{\text{control}} - A_{540}^{\text{sample}} \times 100}{A_{540}^{\text{control}}}$$

(A₄₀₅^{control}, the absorbance of the test without extract; A₄₀₅^{sample}, the absorbance of the test in the presence of extract)

Hydroxyl radical (OH) scavenging activity

Hydroxyl radical scavenging ability of the extract was measured according to the method of Klein et al (16). The extract (0.2 mL) was mixed with 1 mL iron-EDTA solution (0.13% ferrous ammonium sulfate in 0.26% EDTA), 0.5 mL 0.018% EDTA and 1 mL DMSO solution (0.85% in 0.1 mol/L phosphate buffered saline pH 7.4). The reaction was terminated by the addition of 1 mL trichloroacetic acid. Then, 3 mL Nash reagent (7.5 g ammonium acetate, 0.3 mL glacial acetic acid, 0.2 mL acetylacetone, and distilled water-100 mL) was added to the above mixture and incubated at room temperature for 15 minutes. The mixture was read at 412 nm with a UV-visible spectrophotometer. The hydroxyl radical scavenging ability was expressed as mg/100 g of dried

sample using gallic acid as standard.

Hydrogen peroxide (H₂O₂) radical scavenging ability

The ability of the extract to scavenge hydrogen peroxide was determined according to the protocol of Ruch et al (17). Hydrogen peroxide (40mM) was prepared in phosphate buffer (pH 7.4). The extract, dissolved in distilled water, was mixed with hydrogen peroxide solution (0.6 mL, 40mM). Hydrogen peroxide absorbance was determined 10 minutes later against a solution containing the phosphate buffer without H₂O₂, and read at 230 nm. The hydrogen peroxide radical scavenging ability was expressed as mg/100 g of the dried sample using gallic acid as standard.

In vitro carbohydrate-hydrolyzing enzymes inhibitory activity assays

α -Glucosidase (EC 3.2.1.20) inhibitory assay

The α -glucosidase inhibitory activity was determined according to the method described by Ademiluyi and Oboh (18), with slight modifications. Briefly, 250 μ L of the extract at different concentrations (1–5 mg/mL) was incubated with 500 μ L α -glucosidase (1.0 U/mL) solution in 100 mmol/L phosphate buffer (pH 6.8) at 37°C for 15 minutes. Thereafter, 250 μ L pNPG solution (5 mmol/L) in 100 mmol/L phosphate buffer (pH 6.8) was added, and the mixture was further incubated at 37°C for 20 minutes. The absorbance of the released *p*-nitrophenol was measured at 405 nm, and the activity was expressed as percentage (%) control inhibition.

$$\alpha\text{-glucosidase inhibition (\%)} = \frac{A_{405}\text{control} - A_{405}\text{sample} \times 100}{A_{405}\text{control}}$$

(A₄₀₅ control = absorbance of the test without extract; A₄₀₅ sample = absorbance of the test in the presence of extract).

α -Amylase (EC 3.2.1.1) inhibitory assay

The α -amylase inhibitory activity was determined according to the method described by Shai et al (19), with slight modifications. A volume of 250 μ L of the extract at different concentrations (1–5 mg /mL) was incubated with 500 μ L porcine pancreatic amylase (2 U/mL) in 100 mmol/L phosphate buffer (pH 6.8) at 37°C for 20 minutes. 250 μ L of 1% starch dissolved in 100 mmol/L phosphate buffer (pH 6.8) was then added to the reaction mixture and incubated at 37°C for 1 hour. One milliliter of DNSA color reagent was then added and boiled for 10 minutes. The absorbance of the resulting mixture was measured at 540 nm and the inhibitory activity was expressed as percentage (%) control inhibition.

$$\alpha\text{-Amylase inhibition (\%)} = \frac{A_{540}\text{control} - A_{540}\text{sample} \times 100}{A_{540}\text{control}}$$

(A₅₄₀ control, absorbance of the test without extract;

A₄₀₅ sample, absorbance of the test in the presence of extract).

Determination of IC₅₀

The concentration of extract required to cause 50% inhibition (IC₅₀) was calculated by linear regression curve derived from graph of various percentage (%) inhibitions against different concentrations of the extract used.

Data analyses

Data were analyzed using one-way analysis of variance (ANOVA) followed by the Duncan multiple range test and graphical representation of results by GraphPad Prism 5 program (GraphPad Software, San Diego, CA, USA). Statistical significance was considered at *P* value <0.05, with results expressed as mean \pm SD (n=3).

Results

In vitro antioxidant properties of ethanolic extract of *M. oleifera* flower

As shown in Figure 1, the extract revealed a significant (*P*<0.05) inhibitory activity against DPPH in a concentration-dependent manner. However, ethanolic extract of *M. oleifera* flower at different concentrations (10–50 mg/mL) exhibited strong inhibition against DPPH in a dose-dependent manner (IC₅₀ = 15.89 mg/mL) (Table 1), while BHT at the same concentration had IC₅₀ = 5.47

Figure 2 shows the reductive capacities of ethanolic extract of *M. oleifera* flower and gallic acid against ferric, OH radical activity, and hydrogen peroxide inhibition. Ethanolic extract of *M. oleifera* flower showed a very high ferric reducing power (101.76 mg/mL) and moderately high OH free radical scavenging (71.62 mg/mL) and hydrogen peroxide (15.33 mg/mL) inhibitory activities. However, gallic acid showed relatively higher ferric reducing power (118.91 \pm 1.15), OH free radical scavenging (92.21 \pm 1.01), and hydrogen peroxide (20.11 \pm 0.98 mg/mL) inhibitory activities.

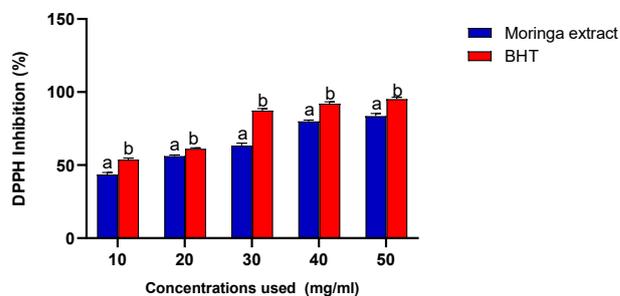


Figure 1. DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging ability of ethanolic extract of *M. oleifera* flowers. Results are represented as mean of triplicate determinations \pm SD (n=3). In each concentration, different alphabets (a and b) on bars represent statistically significant (*P*<0.05) difference. Note: BHT: Butylated hydroxytoluene.

Table 1. IC₅₀ values of the ethanolic extract of *M. oleifera* flower on α -amylase, α -glucosidase and DPPH

	α -Amylase (mg/mL)	α -Glucosidase (mg/mL)	DPPH (mg/mL)
Ethanolic extract of MOF	37.33	38.30	15.89
Acarbose	13.63	23.94	-
Gallic acid	-	-	5.47

Results are represented as mean of triplicate determinations \pm SD (n=3). MOF: *M. oleifera* flower; DPPH: 1,1-diphenyl-2-picrylhydrazyl.

In vitro enzyme inhibitory activity

As shown in Figure 3, the extract revealed a significant ($P < 0.05$) inhibitory activity against α -glucosidase enzyme in a concentration-dependent manner. However, the percentage inhibition varied from 43.21 ± 0.21 to $88.20 \pm 0.99\%$ from the lowest to the highest concentration (IC₅₀ = 38.30 mg/mL) (Table 1). Thus, acarbose at the concentrations of 20-100 mg/mL showed α -glucosidase inhibitory activity from 51.60 ± 0.46 to $93.10 \pm 1.11\%$ (IC₅₀ = 23.94 mg/mL).

As shown in Figure 4, the extract revealed a significant ($P < 0.05$) inhibitory activity against α -amylase enzyme in a concentration-dependent manner. However, ethanolic extract of *M. oleifera* at different concentrations (20-100 mg/mL) exhibited strong inhibition against α -amylase inhibitory activity in a dose-dependent manner (IC₅₀ = 37.63 mg/mL) (Table 1). Acarbose at the concentrations of 20-100 mg/mL showed α -amylase inhibitory activity from $56.61 \pm 1.18\%$ to $95.20 \pm 1.57\%$ with IC₅₀ = 13.63 mg/mL.

Discussion

Polyphenolic compounds have been implicated as a possible bioactive antidiabetic agents (20). In living systems, free radicals are constantly generated and can cause extensive damage to tissues and biomolecules, leading to various disease conditions, especially metabolic disorders (21). The use of DPPH to estimate the activity of antioxidants has recently been reviewed (22). Recent studies have implicated the consumption of natural antioxidants from food as an alternative in the control of free radical activities (23,24). In the present study (Figure 1), the antioxidant capacity of ethanolic extract of dried flower of *M. oleifera* was measured via DPPH inhibition and there was a promising concentration-dependent inhibitory ability of free radicals by ethanolic extract of dried flower of *M. oleifera*.

Hydroxyl radicals are the major active oxygen species causing lipid peroxidation and enormous biological damages (25). However, as demonstrated in this study (Figure 2), incubation of ferric-EDTA with ascorbic acid and H₂O₂ at pH 7.4, in the presence of 2-deoxyribose generated an MDA-like product with resultant pink chromogen upon heating with TBA (26), which was sufficiently inhibited at the introduction of ethanolic extract of dried flowers of *M. oleifera*. Similarly, inhibition

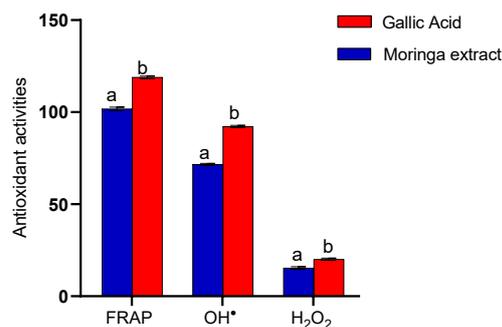


Figure 2. Ferric reducing antioxidant power (FRAP; 100 mg/g dried sample), hydroxyl radical (OH^{*}; 100 mg/g dried sample) scavenging activity and hydrogen peroxide (H₂O₂; 100 mg/g dried sample) scavenging ability of ethanolic extract of *M. oleifera* flower. In each concentration, different alphabets (a&b) on bars represent statistically significant ($P < 0.05$) difference.

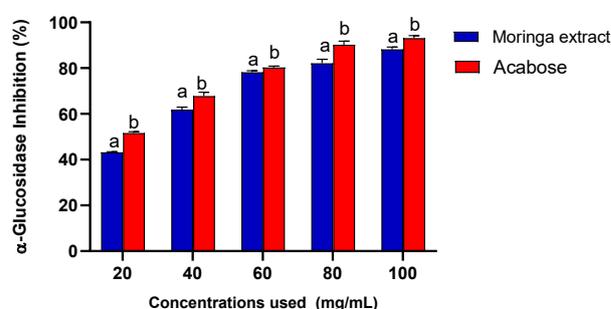


Figure 3. α -Glucosidase inhibitory activity of ethanolic extract of *M. oleifera* flower. Results are represented as mean of triplicate determinations \pm SD (n = 3). In each concentration, different alphabets (a&b) on bars represent statistically significant ($P < 0.05$) difference.

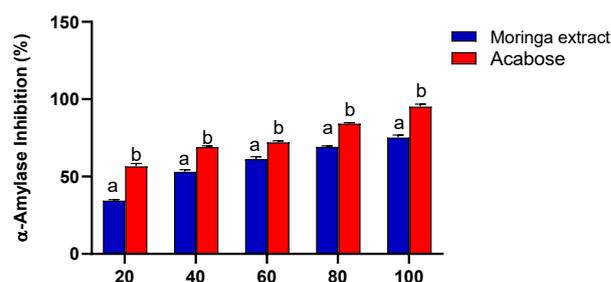


Figure 4. α -Amylase inhibitory activity of ethanolic extract of *M. oleifera* flower. Results are represented as mean of triplicate determinations \pm SD (n = 3). In each concentration, different alphabets (a&b) on bars represent statistically significant ($P < 0.05$) difference.

of hydrogen peroxide (H₂O₂) generating species has been emphasized in recent studies, due to its ability to penetrate biological membranes and produce hydroxyl radical (27). Thus, removing it is paramount in the protection of the biological system (28). Moreover, ROS can react with most biomolecules such as protein, lipids,

and DNA thereby causing oxidative damage, which also plays a significant pathological role in human diseases (29,30). Antioxidants can counteract the oxidation process via various mechanisms, including reacting with free radicals, chelating catalytic metals, oxygen scavenging, decomposition of peroxides, and preventing chain initiation (27,31-33). As shown in this study (Figure 2), ethanolic extract of the dried flower of *M. oleifera* demonstrated an inhibitory potential similar to that of a known standard. As revealed in this study, H₂O₂-scavenging ability of ethanolic extract of the flower of *M. oleifera* may be attributed to the presence of polyphenolic groups as recently reported by Arise et al (34), through donating electrons to H₂O₂, thereby neutralizing it into water.

Furthermore, the reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (35). More so, the activities of antioxidants have been attributed to various mechanisms such as prevention of chain initiation, decomposition of peroxides, reducing capacity, and radical scavenging ability (31). With the results observed in this study, the reducing power of ethanolic extract of dried flower of *M. oleifera* was lower compared to the standard. However, this result indicates that ethanolic extract of dried flower of *M. oleifera* contains significant amounts of flavonoids and phenolic compounds (34). These compounds have been reported to show good antioxidant potential on human nutrition and health considerably (36). Phenolic compounds are very important plant constituents due to their hydroxyl group's confer-scavenging ability (37).

In the management of DM, inhibition of key enzymes involved in the breakdown of carbohydrate (α -amylase and α -glucosidase) leads to inhibition of starch hydrolysis resulting into a decrease in glucose available for assimilation into the blood (regulating postprandial glycemic level) (38). Several *in vitro* studies have confirmed the inhibitory potential of medicinal plants on α -amylase and α -glucosidase activities; whereas, in some cases, the bioactive compounds responsible for this mechanism of action have been identified (39-41). In this study (Figures 3 and 4), the ethanolic extract of *M. oleifera* flower showed a reliable inhibitory effect on α -glucosidase and α -amylase enzymatic activities in a concentration-dependent trend. This observation was as well compared favorably with a known synthetic inhibitor. The inhibition of α -amylase and α -glycosidase enzymes by ethanolic extract of dried flower of *M. oleifera* in the present study provides a strong biochemical basis for its use in the management of DM.

Conclusion

This study has revealed various biological activities of ethanolic extract of *M. oleifera* flower. Ethanolic extract of *M. oleifera* flower demonstrated a promising therapeutic potentiality through free radical scavenging ability and

inhibitory activities on α -amylase and α -glucosidase which could be considered as an alternative phytotherapy in the management of NIDDM. Therefore, isolation, purification, and characterization of compounds responsible for these acclaimed activities of *M. oleifera* flower are suggested for further studies.

Acknowledgment

The authors hereby appreciate the staff of Biochemistry Unit, Afe Babalola University for their support during this study.

Authors' contributions

OOR conceptualized the research and prepared the draft of manuscript; AOB reviewed the literature, analyzed the data, and prepared the draft; OBO conducted the research and analyzed the data; OOA conducted the research and analyzed the data. All authors read and approved the final report.

Conflict of interest

No conflict of interest was declared by the authors.

Ethical considerations

Ethics approval was obtained from the Afe Babalola University ethical committee (Ethical code: ABUAD/ACA/458).

Funding/Support

No financial support was received from governmental/NGO body

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