



Original Article

 Enzymes inhibitory and radical scavenging potentials of two selected tropical vegetable (*Moringa oleifera* and *Telfairia occidentalis*) leaves relevant to type 2 diabetes mellitus


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ABSTRACT

Moringa oleifera Lam., *Moringaceae*, and *Telfairia occidentalis* Hook. f., *Cucurbitaceae*, leaves are two tropical vegetables of medicinal properties. In this study, the inhibitory activities and the radical scavenging potentials of these vegetables on relevant enzymes of type 2-diabetes (α -amylase and α -glucosidase) were evaluated *in vitro*. HPLC-DAD was used to characterize the phenolic constituents and Fe^{2+} -induced lipid peroxidation in rat's pancreas was investigated. Various radical scavenging properties coupled with metal chelating abilities were also determined. However, phenolic extracts from the vegetables inhibited α -amylase, α -glucosidase and chelated the tested metals (Cu^{2+} and Fe^{2+}) in a concentration-dependent manner. More so, the inhibitory properties of phenolic rich extracts from these vegetables could be linked to their radical scavenging abilities. Therefore, this study may offer a promising prospect for *M. oleifera* and *T. occidentalis* leaves as a potential functional food sources in the management of type 2-diabetes mellitus.

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Introduction

In recent years, studies on “Type 2-diabetes mellitus (T2DM)” and its adverse effects on human health have become a subject of considerable interest. T2DM is a chronic metabolic disorder that continues to present a major worldwide life threatening problem. It is characterized by absolute or relative deficiencies in insulin secretion and/or insulin action associated with chronic hyperglycemia and disturbances of carbohydrate, lipid, and protein metabolism. As a consequence of the metabolic derangements in diabetes, various complications develop including both macro and micro-vascular dysfunctions (Duckworth, 2001). One major therapeutic approach for the management of diabetes is to decrease postprandial hyperglycaemia. This is done by retarding or inhibiting absorption of glucose through the inhibition of the key enzymes linked with T2DM, α -amylase and α -glucosidase, (carbohydrate-hydrolyzing enzymes) in the digestive tract. Consequently, inhibitors of these key enzymes determine a reduction in the rate of glucose absorption and consequently blunting the post-prandial plasma glucose rise (Chiasson, 2006; Chen et al., 2006).

However, it is very hapless that the pharmaceutical drugs formulated for the inhibition of these key enzymes always come with attendant side effects coupled with their expensive cost (Adefegha and Oboh, 2012). Hence, a search for a cheap alternative management approach with little or no side effect becomes pertinent. Meanwhile, recent studies on the beneficial health effects of plants have raised the interest of researchers on the possible preventive and protective actions of plants against chronic diseases (Udenigwe et al., 2012). As phenolics are a class of chemical compounds found in plant foods; therefore, their reported roles in mediating α -amylase inhibition could however, offer a promising management strategy for Type 2-diabetes (Cheplick et al., 2010).

Furthermore, compounds that offer health benefiting potentials are embedded in vegetables; the main protective action of vegetables has been attributed to the presence of antioxidants, especially antioxidant vitamins which include ascorbic acid, α -tocopherol, β -carotene and phenolics (Oboh and Rocha, 2007). However, various prospective studies have revealed that the majority of antioxidant activities may be from compound such as: catechin, isocatechin, flavonoids, flavones, anthocyanin, isoflavone and rather than vitamins C, E and β -carotene (Oboh and Rocha, 2007). Several green leafy vegetables with high phenolic contents abound in the tropical region of Africa and they are utilized either as condiments or spices in human diets (Akindahunsi and Oboh, 1999).

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Kumar et al. (2010) have reported the medicinal value of *Moringa* and related it to its roots, bark, leaves, flowers, fruits, and seeds. *Moringa oleifera* Lam., Moringaceae and *Telfairia occidentalis* Hook f., Cucurbitaceae, a fluted pumpkin are two selected green leafy vegetables widely consumed in Nigeria. Leaves from these vegetables constitute an important ingredient in soup making. *Moringa oleifera* leaf has anti-cancer (Guevara et al., 1999), anti-inflammatory (Kurma and Mishra, 1998), hypoglycemic potential (Kar et al., 2003), and its thyroid status regulator has been well investigated by (Talhiani and Kar, 2000). Extracts from these vegetables have been shown to possess antidiabetic activity in both alloxan and streptozotocin diabetic animals (Nwozo et al., 2004). In many regions of Africa, *M. oleifera* leaves are widely consumed for self-medication by patients affected by diabetes and hypertension (Kasolo et al., 2010; Monera and Maponga, 2010).

Moreover, several findings have been carried out on the chemical characterization of phytoconstituents and antidiabetic properties of tropical green leafy vegetables, yet there is still dearth of information in respect of the possible mechanism by which they offer their antidiabetic properties. To this end, this study sought to investigate the enzymes inhibitory and the radical scavenging potentials of *M. oleifera* and *T. occidentalis* leaves on key enzyme relevant to type-2 diabetes (α -amylase and α -glucosidase) in order to establish some critical underline mechanisms by which they are used in the management of type 2-diabetes.

Materials and methods

Materials

Telfairia occidentalis Hook f., Cucurbitaceae, and *Moringa oleifera* Lam., Moringaceae, leaves were purchased from an ancient Oja Oba market in Owo Kingdom, Ondo State Nigeria. Authentication of the vegetables was carried out by Omotayo F. O of the Plant Science and Biotechnology Department, Ekiti State University, Ado, Ekiti (EKSU) Nigeria (Herbarium numbers: UHAE. 2016/084 and UHAE. 2016/085) for *T. occidentalis* and *M. oleifera* leaves respectively. The leaves were separated from their stems and subsequently washed under running tap water, sun dried and triturated.

Chemicals and reagents

All chemicals and reagents used in this study were of analytical grade and were obtained from standard commercial suppliers.

Experimental animals

Thirty two male adult Wistar rats (200–250 g) from our own breeding colony were used. Animals were kept in separate animal cages, on a 12 h light:12 h dark cycle, at a room temperature of 22–24 °C, and with free access to food and water. They were acclimatized under these conditions for two weeks prior to the commencement of the experiments. The animals were used according to standard guidelines of our university (Protocol No. 200141).

Preparation of the extracts

Each triturated samples (20 g) was homogenized with 100 ml of distilled water. The homogenate was filtered through Whatman (No. 2) filter paper and later centrifuged at 2000 × g for 10 min to obtain clear supernatant. The supernatant was then freeze dried into powdered form with the aid of freeze drier. Each freeze dried extracts (2 g) was then dissolved into 100 ml of distilled water, stored at 4 °C and used for subsequent analysis. The freeze dried samples were used for HPLC-DAD analysis.

Preparation of rat's pancreas for the experiments

Male adult Wistar rats were incapacitated via cervical dislocation and the pancreas was rapidly separated, rinsed with cold saline, placed on ice and weighed. This tissue was subsequently rinsed in cold saline solution and later homogenized in phosphate buffer pH 7.4 (1:5 w/v) with about 10-up and-down strokes at approximately 1200 rev/min in a Teflon-glass homogenizer. The homogenate was centrifuged for 10 min at 3000 × g to yield a pellet that was discarded and the supernatant was used for lipid peroxidation assay (Belle et al., 2004).

Determination of total phenol content

The total phenol content was determined according to the method of (Singleton et al., 1999).

Determination of total flavonoid content

The total flavonoid content was determined using a slightly modified method reported by (Meda et al., 2005).

Determination of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging ability

The free radical scavenging ability of the sample extracts against DPPH (1,1-diphenyl-2 picrylhydrazyl) free radical was evaluated as described by (Gyamfi et al., 1999).

Determination of reducing property

The reducing property of the extracts was determined by assessing the ability of the extracts to reduce FeCl₃ solution as described by (Oyaizu, 1986).

Copper (Cu²⁺) chelation assay

Copper chelating ability was measured by the methods of Torres-Fuentes et al. (2011).

Fe²⁺ chelation assay

The Fe²⁺ chelating ability of the extracts were determined using a modified method of Minnoti and Aust (1987) with a slight modification by Puntel et al. (2005).

Lipid peroxidation and thiobarbituric acid reactions

Lipid peroxidation, induced by Fe²⁺ in isolated rat pancreas homogenates was carried out using the modified method of (Ohkawa et al., 1979).

α -Amylase inhibition assay

Extract (50 μ l) and 500 μ l of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing Hog pancreatic α -amylase (EC 3.2.1.1) were incubated at 25 °C for 10 min. Then, 500 μ l of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added to each tube. The reaction mixtures was incubated at 25 °C for 10 min and stopped with 1 ml of dinitrosalicylic acid colour reagent. Thereafter, the mixture was incubated in a boiling water bath for 5 min, and cooled to room temperature. The reaction mixture was then diluted by adding 10 ml of distilled

water, and absorbance measured at 540 nm in a spectrophotometer (Worthington, 1993).

$$\text{Inhibition (\%)} = \frac{\text{Abs}_{\text{ref}} - \text{Abs}_{\text{sam}}}{\text{Abs}_{\text{ref}}} \times 100$$

where Abs_{ref} is the absorbance without samples extract and Abs_{sam} is absorbance of samples extract.

α -Glucosidase inhibition assay

Appropriate dilution of the extracts (50 μl and 100 μl) of the α -glucosidase solution (EC 3.2.1.20) (0.5 mg/ml) in 0.1 M phosphate buffer (pH 6.9) was incubated at 25 °C for 10 min. Then, the 50 μl of 5 mM *p*-nitrophenyl- α -D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added. The mixtures were incubated at 25 °C for 5 min, before reading the absorbance at 405 nm in the spectrophotometer. The α -glucosidase inhibitory activity was expressed as percentage inhibition (Apostolidis et al., 2007) and it was calculated as follows:

$$\text{Inhibition (\%)} = \frac{\text{Abs}_{\text{ref}} - \text{Abs}_{\text{sam}}}{\text{Abs}_{\text{ref}}} \times 100$$

where Abs_{ref} is the absorbance without samples extract and Abs_{sam} is absorbance of samples extract.

Quantification of compounds by HPLC-DAD

Reverse phase chromatographic analyses were carried out under gradient conditions using C_{18} column (4.6 mm \times 150 mm) packed with 5 μm diameter particles; the mobile phase was water containing 1% formic acid (A) and methanol (B), and the composition gradient was: 13% of B until 10 min and changed to obtain 20, 30, 50, 60, 70, 20 and 10% B at 20, 30, 40, 50, 60, 70 and 80 min, respectively, following the method described by (Pereira et al., 2014) with slight modifications. Sample extracts and mobile phase were filtered through 0.45 μm membrane filter (Millipore) and then degassed by ultrasonic bath prior to use, the sample extracts were analyzed at a concentration of 20 mg/ml. The flow rate was 0.6 ml/min, injection volume 50 μl and the wavelength were 254 for gallic acid, 280 for catechin and epicatechin, 327 nm for chlorogenic, caffeic and ellagic acids, and 365 nm for quercetin, quercitrin, isoquercitrin, rutin and kaempferol. All chromatography operations were carried out at ambient temperature and in triplicate.

Statistical analysis

The mean values from triplicate experiments were pooled and expressed as the mean \pm standard deviation (STD) and one way analysis of variance was used to analyze the results. Tukey's test was used for the post hoc treatment using Statistical Package for Social Science (SPSS) 16.0 for windows. The least significance difference (LSD) was taken at $p < 0.05$. GraphPad Prism 6 software was subsequently used for the IC_{50} (extract concentration causing 50% enzyme inhibition) analysis. Values were determined using non-linear regression analysis.

Results

Leaves extracts from both *M. oleifera* and *T. occidentalis* species inhibited α -amylase activity in a concentration dependent manner (14.29 $\mu\text{g/ml}$) (Fig. 1). However, the IC_{50} (sample concentration inhibiting 50% enzyme activity) values (Table 1) revealed that *M. oleifera* leaf (6.49 $\mu\text{g/ml}$) had higher enzyme inhibitory activity compare to (10.60 $\mu\text{g/ml}$) *T. occidentalis* leaf. Also, the effect of the two selected tropical vegetables on α -glucosidase activity *in vitro* was investigated (Fig. 2). The results showed

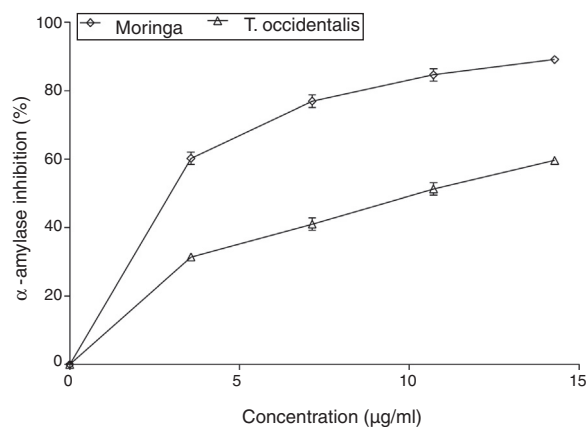


Fig. 1. α -Amylase inhibitory effects of the aqueous extracts from the leaves of *Moringa oleifera* and *Telfairia occidentalis*. Values represent mean \pm standard deviation, $n = 3$.

Table 1

IC_{50} values of the inhibitory activities of two selected tropical vegetables ($\mu\text{g/ml}$) on α -amylase, α -glucosidase, copper chelation, iron chelation FeSO_4 induced lipid peroxidation, OH and DPPH radical scavenging abilities in the rat's pancreas homogenates.

	Samples	
	<i>Moringa oleifera</i> leaf	<i>Telfairia occidentalis</i> leaf
α -Amylase	6.49 \pm 0.07 ^a	10.60 \pm 0.90 ^b
α -Glucosidase	4.73 \pm 0.05 ^b	7.69 \pm 0.06 ^a
Copper chelation	3.14 \pm 0.19 ^a	5.49 \pm 0.13 ^b
Iron chelation	3.23 \pm 0.07 ^b	3.60 \pm 0.04 ^a
Lipid peroxidation	27.85 \pm 0.64 ^a	66.35 \pm 0.12 ^b
OH ^c	18.88 \pm 0.12 ^b	37.58 \pm 1.00 ^a
DPPH ^c	14.18 \pm 0.29 ^a	21.06 \pm 0.82 ^b

Values represent means of triplicate. Values with the different alphabet along the same row are significantly different ($p > 0.05$).

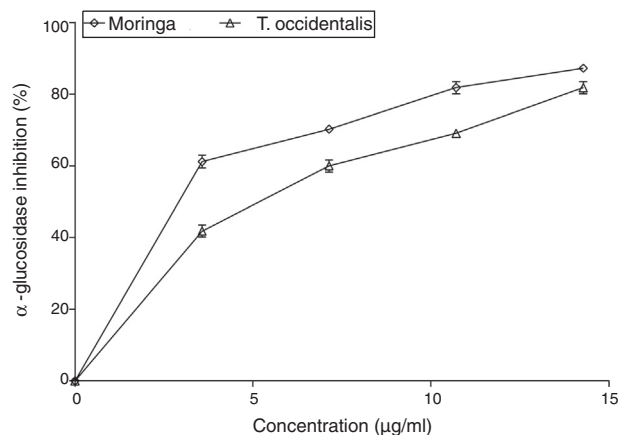


Fig. 2. α -Glucosidase inhibitory effects of the aqueous extracts from the leaves of *Moringa oleifera* and *Telfairia occidentalis*. Values represent mean \pm standard deviation, $n = 3$.

that the vegetables extracts inhibited α -glucosidase activity in a concentration-dependent manner (14.29 $\mu\text{g/ml}$). Nonetheless, as revealed by the IC_{50} values (Table 1), *M. oleifera* leaf (4.73 $\mu\text{g/ml}$), had a significantly ($p < 0.05$) higher α -glucosidase inhibitory activity than *T. occidentalis* leaf (7.69 $\mu\text{g/ml}$).

HPLC-DAD analysis of the phenolic phytoconstituents of the extracts (Table 2) showed that *M. oleifera* leaf had the highest significant ($p < 0.01$) amount of gallic acid, chlorogenic acid, ellagic acid, rutin, quercitrin, isoquercitrin, quercetin and kaempferol while

Table 2
Phenolics phytoconstituents of the two selected vegetables *Moringa* and *Telfairia occidentalis* leaves extracts as revealed by HPLC-DAD analysis.

Compounds	<i>Moringa</i> leaf	<i>T. occidentalis</i> leaf	LOD	LOQ
	(mg/g)			
Gallic acid	32.45 \pm 0.02 ^a	0.93 \pm 0.02 ^a	0.009	0.034
Catechin	9.98 \pm 0.01 ^b	13.91 \pm 0.15 ^a	0.031	0.102
Chlorogenic acid	50.69 \pm 0.03 ^c	1.32 \pm 0.03 ^b	0.017	0.056
Caffeic acid	6.28 \pm 0.03 ^d	7.44 \pm 0.01 ^a	0.026	0.089
Ellagic acid	33.15 \pm 0.02 ^a	2.37 \pm 0.02 ^d	0.011	0.037
Epicatechin	10.03 \pm 0.01 ^b	15.48 \pm 0.03 ^c	0.025	0.084
Rutin	15.27 \pm 0.02 ^e	5.74 \pm 0.01 ^b	0.019	0.063
Quercitrin	29.74 \pm 0.01 ^a	6.42 \pm 0.02 ^e	0.038	0.125
Isoquercitrin	64.53 \pm 0.03 ^e	7.35 \pm 0.01 ^f	0.007	0.024
Quercetin	47.91 \pm 0.01 ^a	5.27 \pm 0.02 ^b	0.024	0.080
Kaempferol	18.23 \pm 0.01 ^f	9.46 \pm 0.03 ^d	0.021	0.069

Results are expressed as mean \pm standard deviations (SD) of three determinations. Averages followed by different letters differ by Tukey test at $p < 0.01$.

Table 3
Total phenol and total flavonoid contents of aqueous extracts from *Moringa* and *Telfairia occidentalis* leaves.

Samples	Total phenol (mg/GAE g)	Total flavonoid (mg/QUE g)
<i>Moringa oleifera</i> leaf	29.20 \pm 0.40 ^a	17.48 \pm 0.18 ^b
<i>T. occidentalis</i> leaf	16.04 \pm 0.23 ^b	10.49 \pm 0.51 ^c

Values represent means \pm standard deviation of triplicate readings. Values with the same superscript letter on the same column are not significantly different ($p > 0.05$).

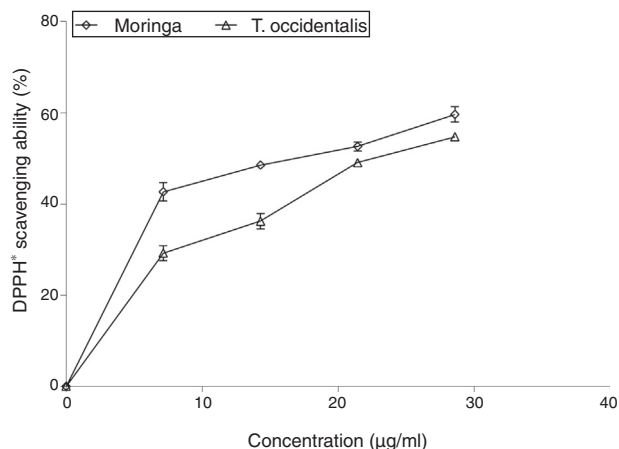


Fig. 3. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging ability of the aqueous extracts from the leaves of *Moringa oleifera* and *Telfairia occidentalis*. Values represent mean \pm standard deviation, $n = 3$.

catechin, caffeic acid and epicatechin had the highest amount of phenolics in *T. occidentalis* leaf.

Furthermore, Table 3 represents the total phenol content of the extracts from *M. oleifera* leaf which ranged from 16.04 (mg/GAE g) (*T. occidentalis* leaf) to 29.20 (mg/GAE g) (*M. oleifera* leaf) while the flavonoid content of the extracts ranged from 10.49 (mg/QUE g) (*T. occidentalis* leaf) to 17.48 (mg/QUE g) (*M. oleifera* leaf).

The free radical scavenging ability of the leaves extracts were also assessed and the IC_{50} as presented in (Table 1) revealed that both leaves extracts scavenged DPPH radical in concentration dependent manner (0–28.57 $\mu\text{g/ml}$) (Fig. 3). However, as revealed by the IC_{50} values, *M. oleifera* leaf had higher scavenging ability (14.18 $\mu\text{g/ml}$) compare to *T. occidentalis* leaf (21.06 $\mu\text{g/ml}$). More so, the OH^{\bullet} scavenging ability of the vegetables extracts were as well determined and this revealed the OH^{\bullet} scavenging ability in a concentration dependent manner (0–28.57 $\mu\text{g/ml}$). Similarly, the extracts from the vegetables chelated Cu^{2+} and Fe^{2+} in a

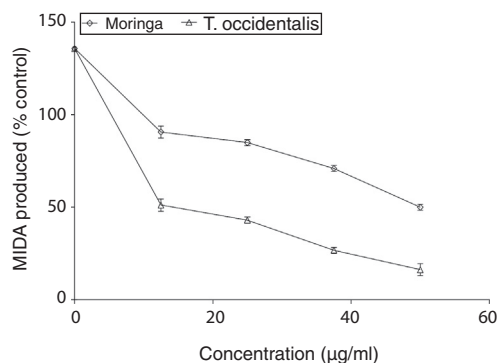


Fig. 4. Inhibition of Fe^{2+} induced lipid peroxidation in rat pancreatic tissue homogenate by the aqueous extracts from the leaves of *Moringa oleifera* and *Telfairia occidentalis*. Values represent mean \pm standard deviation, $n = 3$.

concentration dependent manner (7.14 $\mu\text{g/ml}$) with the IC_{50} value (3.14 $\mu\text{g/ml}$) *M. oleifera* leaf having higher Cu^{2+} chelating ability than *T. occidentalis* leaf (5.49 $\mu\text{g/ml}$), while there was a significant ($p < 0.05$) difference in the Fe^{2+} chelating ability between *M. oleifera* leaf (3.23 $\mu\text{g/ml}$) and *T. occidentalis* leaf (3.60 $\mu\text{g/ml}$).

Table 1 revealed the ferric reducing antioxidant power (FRAP) of the extracts from the two selected tropical vegetables, presented as ascorbic acid equivalent. Both extracts displayed ferric reducing antioxidant power; (16.89 mg AAE/g) *M. oleifera* leaf had higher reducing power than (8.44 mg AAE/g) *T. occidentalis* leaf. More so, incubation of pancreas homogenates in the presence of Fe^{2+} caused a significant increase ($p < 0.05$) in MDA content. However, extracts from the two selected tropical vegetables inhibited MDA production (Fig. 4) with the IC_{50} (27.85 $\mu\text{g/ml}$) *M. oleifera* leaf revealing higher inhibitory activity compared to (66.35 $\mu\text{g/ml}$) *T. occidentalis* leaf (Table 1).

Discussion

Medicinal plants with anti-diabetic properties have been reported to offer promising potentials of enzyme inhibition (Wadkar et al., 2008). However, the inhibitory activity exhibited by *M. oleifera* and *T. occidentalis* leaves on the key enzymes relevant to diabetes mellitus (α -amylase and α -glucosidase) in this study (Figs. 1 and 2) could be one of the mechanisms in which the extracts cause reduction in blood glucose level. α -Amylase is one of the key enzymes involved in the break down of starch into absorbable glucose molecules (Affi et al., 2008). Inhibiting these enzymes help in reducing post-prandial glycemia by reducing the speed of glucose absorption (Sangeetha and Vedasree, 2012). It is remarkable that *M. oleifera* leaf exhibited higher α -amylase inhibitory activity than *T. occidentalis* leaf, this result could be attributed to its higher phenolics content (Table 3). More so, as revealed by the IC_{50} values (extract concentration causing 50% enzyme inhibition) *M. oleifera* leaf (6.49 $\mu\text{g/ml}$) had significantly ($p < 0.05$) higher α -amylase inhibitory activity than *T. occidentalis* leaf (10.60 $\mu\text{g/ml}$) (Table 1).

Nonetheless, this inhibitory activity of the two selected tropical vegetables agrees with a previous report where *M. oleifera* leaf was used in animal model of diabetes (Jaiswal et al., 2009). In the same vein, the ability of the two selected vegetable extracts to inhibit α -glucosidase activity was also experimented and the result is presented in (Fig. 2). The result revealed that both vegetable extracts inhibited α -glucosidase in a concentration dependent manner (14.29 $\mu\text{g/ml}$). Nonetheless, as revealed by the IC_{50} values (Table 1), *M. oleifera* leaf (4.73 $\mu\text{g/ml}$), had a significantly ($p < 0.05$) higher α -glucosidase inhibitory activity than *T. occidentalis* leaf (7.69 $\mu\text{g/ml}$). This result agrees with the previous findings of Kumari (2010)

and Giridhari et al. (2011) where *M. oleifera* leaf exhibited higher antidiabetes properties. Meanwhile, current data has revealed that phenols are potential inhibitors of α -amylase and α -glucosidase activities (Obboh et al., 2015). This result could however, suggest that the inhibition of α -amylase and α -glucosidase activities by these two selected tropical vegetables may be due to their phenolic phytoconstituents (Obboh et al., 2015). It is noteworthy that this evince is in agreement with previous study where inhibition of α -amylase and α -glucosidase by medicinal plants has been suggested to possess promising management strategy for type 2 Diabetes mellitus (T2DM) coupled with their critical bioactive compounds such as polyphenols that may offer valuable structure–function benefits (Kwon et al., 2007).

Furthermore, the results of this current research revealed the presence of large amount of phenols and flavonoids in both tested tropical vegetable extracts (Table 3). However, *M. oleifera* leaf had a significantly ($p < 0.05$) higher total phenol (29.2 mg/GAE g) and flavonoid (17.48 mg/QUE g) content than *T. occidentalis* leaf total phenol (16.04 mg/GAE g) and flavonoid (10.49 mg/QUE g) content. This finding reflects the potency of *M. oleifera* leaf over *T. occidentalis* leaf as it is consistent with previous study that has established a strong correlation between the phenolic contents and antioxidant properties of plant foods (Chu et al., 2002). More so, Table 2 depicts the higher abundance of phytoconstituents as revealed by the HPLC-DAD analysis. *M. oleifera* leaf had the highest significant ($p < 0.05$) amount of gallic acid, chlorogenic acid, ellagic acid, rutin, quercitrin, isoquercitrin, quercetin and kaempferol while catechin, caffeic acid and epicatechin had the highest amount of phenolics in *T. occidentalis* leaf. Phenolic compounds are very essential secondary metabolites in plants and are reported to be responsible for the variation in antioxidant activities in plants (Uyoh et al., 2013). They are as well capable of fighting against free radicals by inactivating free radicals or preventing decomposition of hydrogen peroxide into free radicals. Therefore, this abundant phytoconstituents in the vegetable extracts could be one of the reasons for their employment in the management of diabetes as reported in folklore (Dieye et al., 2008).

The chemical structure of iron and its capacity to drive a one-electron reaction makes it a key factor in the formation of free radicals (Fraga and Oteiza, 2002). However, a study by Ikpeme et al. (2014) affirmed the damaging effects mediated by free radicals in cells, as their activities in protein denaturation, lipid peroxidation and the disruption of membrane fluidity have been implicated in oxidative stress. Nonetheless, phenolics from plants origin have the metabolic ability to quench these radicals as a result of their modulating power (Anokwuru et al., 2011) while this may in turn help hindering oxidative stress related diseases (Shukla et al., 2009). This study has shown that *M. oleifera* and *T. occidentalis* leaves were able to prevent the progression of lipid peroxidation by inducing a reduction in MDA content of the pancreatic tissues in a concentration dependent manner (Fig. 4). In addition, the IC_{50} values indicated that *M. oleifera* leaf had higher iron induced lipid peroxidation effect than *T. occidentalis* leaf (Table 1). This result accedes with earlier study where inhibitory effects of plant constituents against pro-oxidant induced lipid peroxidation in selected animal tissues were reported (Obboh and Rocha, 2007).

Moreover, the strong radical scavenging ability of the two tropical vegetables observed in this study as exemplified by their reducing power, radical (FRAP, DPPH and OH) scavenging abilities and their metal (Fe^{2+} and Cu^{2+}) chelating properties suggests that they might be good dietary sources of antioxidant. Considering this report, the reducing power of the extracts was measured by their ability to reduce Fe^{3+} to Fe^{2+} . Both extracts exhibited their reducing power by reducing Fe^{3+} to Fe^{2+} while *M. oleifera* leaf had higher reducing power compared to *T. occidentalis* leaf (Fig. 5). Meanwhile, the antioxidant potentials of extracts from plants species have been related with their capacity to reduce oxidative species (Islam, 2013) while this is in line with the work of (Atawodi et al., 2010) where the therapeutic actions of *M. oleifera* leaf was related to the relatively high antioxidant activity of its leaves flowers and seeds. Therefore, this result could be linked to the rich phenolic content of *M. oleifera* leaf as revealed by the HPLC-DAD fingerprinting (Table 2).

Since the antioxidant activities of plants is a function of their phenolics; the DPPH and OH radicals scavenging abilities revealed

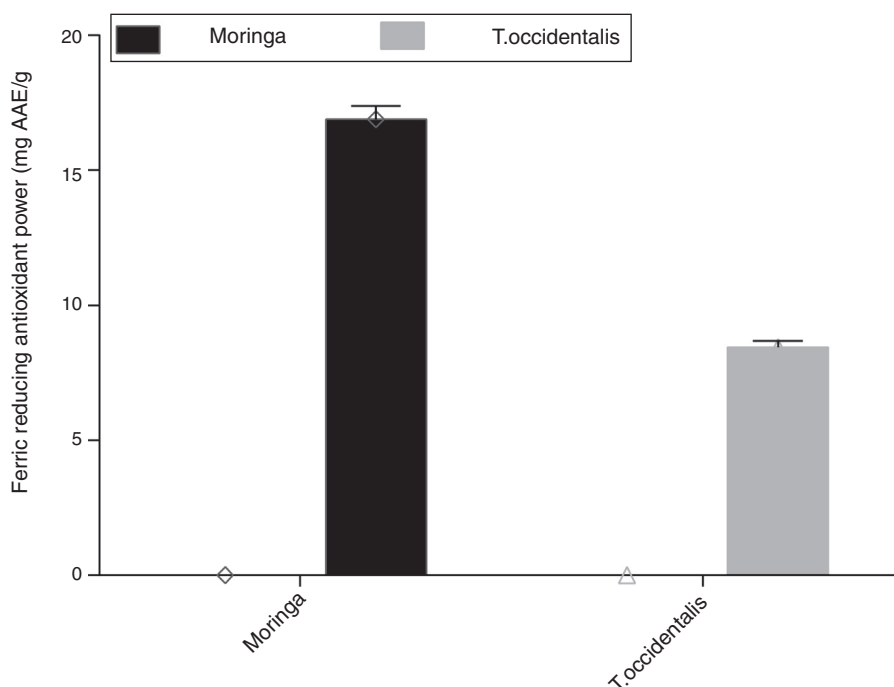


Fig. 5. Ferric reducing antioxidant power (FRAP) of the aqueous extracts from the leaves of *Moringa oleifera* and *Telfairia occidentalis*. Values represent mean \pm standard deviation, $n = 3$.

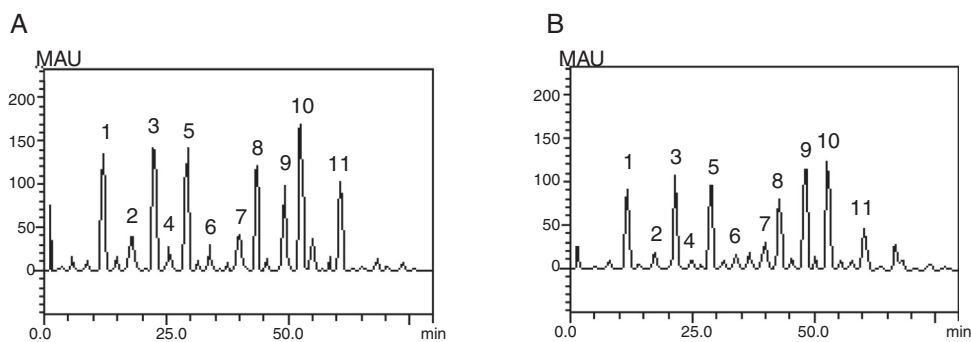


Fig. 6. Represents high performance liquid chromatography profile of (A) *Moringa oleifera* and (B) *Telfairia occidentalis* extracts. Ellagic acid (peak 1), epicatechin (peak 2), chlorogenic acid (peak 3), caffeic acid (peak 4), quercetin (peak 5), catechin (peak 6), rutin (peak 7), gallic acid (peak 8), kaempferol (peak 9), isoquercitrin (10) and quercitrin (peak 11).

by these two vegetables may be due to their phenolic phytoconstituents (Fig. 3 and Table 1). More so, scavenging activity observed on DPPH radical may be due to their hydrogen donating ability to the unstable DPPH free radical that accepts an electron or hydrogen to become a stable diamagnetic molecule (Siddaraju and Dharmesh, 2007). This is worthy of note with the alignment observed in their IC₅₀ values (Table 1). As such, it is probable that the decrease in absorbance of DPPH radical caused by phenolic compound in this study may be as a consequence of the reaction between antioxidant molecules in the extracts and the radicals. Therefore, a correlation with a previous report where some plant extracts exhibited oxidative stress mitigating properties on excessive radical related diseases such as diabetes, cancer and arthritis could be established (Tripathy et al., 2010) (Fig. 6).

In addition, the metal chelating (Cu²⁺ and Fe²⁺) ability of the two tropical vegetables could be one of the mechanisms underlying the inhibitory effect of these leaves on key enzymes relevant to T2DM management/treatment (Table 1). Ghimeray et al. (2009) succinctly reported the critical roles played by transition metals in the generation of free radicals in the biological cells. Meanwhile, Cu²⁺ and Fe²⁺ have the capacity to initiate free radical generation process owing to the unpaired electrons on their valence shells which makes them structurally unstable. These unsteady properties of transition metals is fundamental to their conversion of H₂O₂ to OH via Fenton reaction coupled with their decomposition of alkyl peroxides to heavy reactive alkyl and hydrogen radicals (Hsu et al., 2006).

As most of these enzymes use these biologically relevant transition metals in their active sites as cofactor for catalysis, chelation of these metals by other compounds would elicit an inhibitory effect on the enzyme activity. The metal chelating (Cu²⁺ and Fe²⁺) ability of the two tested tropical vegetables observed in this study agrees with the earlier report by (Finefrock et al., 2003) where plants with antioxidant potentials chelate metal ions and potentially inhibit metal dependent processes by donating electrons to these unstable-electron-deficient metals. Therefore, with phenolics as known metal chelators, there is no doubt that plants with high contents of polyphenols are good radical scavengers and it would not be a mere coincidence to realize that *M. oleifera* leaf with the highest phenolic content/constituents exhibited the highest inhibitory effect on the key enzymes investigated in this study. To this end, the results obtained in this present study may be the basic principle underline the pharmacological evidence in support of the folkloric claim that these tropical vegetables were used as anti-diabetic plants. In conclusion, the ability of the two selected tropical vegetables to scavenge free radicals and inhibit key enzymes relevant to type 2-diabetes mellitus (α -amylase and α -glucosidase) may be due to their high contents of phenolics. These could be some of the possible mechanisms underlying the anti-diabetic properties of these tropical vegetables as reported in folklore. Meanwhile,

M. oleifera leaf appeared to be the most potent of the vegetables investigated and may serve as the basis in the future formulation of functional foods and nutraceuticals for the management/treatment of type 2-diabetes.

Conflicts of interest

The author has declared that no competing interest exist.

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