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Anti- radical and Inhibitory Effect of some Common Nigerian Medicinal Plants on Alpha Glucosidase, Aldose Reductase and Angiotensin Converting Enzyme: Potential Protective Mechanisms against Diabetic Complications

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Abstract

In this study, the methanol extract of the leaves of five common medicinal plants- *Morinda lucida* (MEML), *Alchornea cordifolia* (MEAC), *Anthocleista vogelli* (MEAV), *Cassia sieberena* (MECS) and *Nauclea latifolia* (MENL) were screened for phytochemical composition, total polyphenol/ flavonoid content, antioxidant activity and inhibitory potentials against key enzymes linked to diabetic complications; α-glucosidase, aldose reductase and angiotensin converting enzyme (ACE). Phytochemical screening revealed the presence of alkaloids, phenols, flavonoids, saponins, steroids and tannins in the extracts. The total polyphenolic content of the extracts was found to be 178 ± 2.3, 158 ± 2.3, 100 ± 1.2, 70 ± 1.7 and 120 ± 2.8 (μg/g of Gallic acid equivalent) for MEML, MEAC, MEAV, MECS and MENL respectively while the total flavonoid content was found to be 48 ± 0.9, 40 ± 1.2, 33 ± 0.8, 26 ± 0.5 and 38 ± 0.8 (μg/g of Quercetin equivalent) in the same order. All the extracts exhibited antioxidant activity as well as inhibitory action on α-glucosidase, aldose reductase and angiotensin converting enzyme activities. However, there were variations in the activities of the extracts. *Morinda lucida* showed the highest antioxidant activity and it was also the most potent inhibitor of α-glucosidase activity, *Alchornea cordifolia* was the most potent against the activity of aldose reductase while *Nauclea latifolia* was the most potent inhibitor of ACE activity. It was concluded that these plants individually or as a polyherbal formulation, could be useful in the management of diabetic complications. However, further investigations are recommended.

Keywords: Anti- radical, Alpha Glucosidase, Aldose Reductase, Angiotensin Converting Enzyme, Diabetic Complications

1. Introduction

Diabetes mellitus is a common metabolic disease associated with many microvascular and macrovascular complications. The microvascular complications include nephropathy, retinopathy, and neuropathy while macrovascular complications include coronary artery diseases, myocardial infarction, stroke, peripheral vascular disease as well as diabetic foot ulcers. These complications are responsible for the high morbidity and mortality associated with the disease (Andrew, 2000; Atlan, 2003, Hadler *et al.*, 2003, Merlin *et al.*, 2005; Fowler, 2008). Diabetic nephropathy has been reported to occur in 25– 40% of peoples with type I or type II diabetes and it increases by 6% per year (MacIsaac & Jerums, 2003), where as diabetic neuropathy occurs in 50% to 66% of diabetic patients (Boulton, 2005; Bouton *et al.,* 2005; Hall *et al*., 2005; Argoff *et al.*, 2006), while diabetic retinopathy with 5% is the fifth leading cause of blindness worldwide (WHO, 2006). Hyperglycemia play an important role in the pathogenesis of diabetic complications by several mechanisms such as increased aldose reductase (AR)-related polyol pathway flux, increased formation of advanced glycation end products (AGE) formation and excessive oxidative stress. Therefore the effective control of blood glucose level is the key to preventing the diabetic complications for both Type I and Type II diabetic patients (DeFronzo, 1999).

Hyperglycaemia is currently managed through the use of insulin and oral hypoglycemic agents such as sulfonylureas, biguanides, meglitinides, thiazolidinediones and incretin mimetics. Unfortunately, none of these therapeutic agents has been completely successful in controlling the long-term microvascular and macrovascular complications. Consequently, there is a strong preference for medicinal plants which are believed to be suitable for managing these complications. The medicinal values of plants lie in their component phytochemicals such as alkaloids, tannins, flavonoids and other phenolic compounds, which produce a definite physiological action on the human body. A systematic search for useful bioactivities from medicinal plants is now considered to be a rational approach in nutraceutical and drug research. This study was aimed at examining some of the physiological actions of plants that are beneficial to the management of diabetic complications. These include antioxidant activity and the ability to modulate the activities of key enzymes (α- Glucosidase, aldose reductase and ACE) that are linked to diabetic complications.

It has been demonstrated that diabetic patients are under oxidative stress; the elevation of free-radical generation and decline in the antioxidant defense may partially mediate the beginning and progression of diabetes associated complications (Jin *et al*., 2008; Vos *et al*., 2012). Several hypothesis have been put forth to explain the genesis of free radicals in diabetes. Formation of excess superoxide radicals by the mitochondrial transport chain during hyperglycemia has been reported to be the initial factor. Therefore, use of antioxidants can be beneficial for diabetic patients, not only to maintain antioxidants levels in the body but also to treat the long term complications that can arise (Iwai, 2008). The polyphenolic compounds in edible plants are currently regarded as natural antioxidants, and their antioxidant activities are therefore important in the management of diabetes and its complications.

Hydrolysis of dietary carbohydrates such as starch is the major source of glucose in the blood. Intestinal α-glucosidase catalyzes the final step in the digestive process of carbohydrates. The inhibition of these enzymes significantly decreases digestion and uptake of carbohydrates and lowers the postprandial blood glucose level in the non-insulin dependent diabetes mellitus patients (Fred- Jaiyesimi *et al.,* 2009). α-Glucosidase inhibitors such as acarbose, miglitol, and voglibose are known to reduce postprandial hyperglycemia primarily by interfering with the carbohydrate digestive enzymes and by delaying glucose absorption. Some flavonoids and polyphenols as well as sugar derivatives are found to be effective in inhibiting α-glucosidase and (Haraguchi *et al.,* 1996; Lee and Kim, 2001) therefore, much effort has been focused on plants to produce potentially useful products such as commercial α-glucosidase inhibitors or lead compounds.

Aldose reductase is the first enzyme in the polyol pathway; it catalyzes the reduction of D- glucose from the aldehyde form into D- sorbitol with concomitant conversion of NADPH to $NADP^+$ (Kador *et al.,* 1985b). It is generally accepted that this polyol pathway plays an important role in the development of some degenerative complications of diabetes. The elevated blood glucose level, characteristic of diabetes mellitus, causes significant fluxes of glucose through the polyol pathway in tissues such as nerves, retina, lens, and kidneys, where glucose uptake is independent of insulin (Chihiro, 1998).

Thus, AR inhibitors have attracted attentions in therapeutic researches of diabetic complications. Several authors have studied and reported on a number of structurally diverse naturally occurring and synthetic AR inhibitors that have proven to be effective for the prevention of diabetic complications in experimental animals, as well as in clinical trials (Guzmán & Guerrero, 2005; Patel *et al.*, 2012).

Previous reports have revealed that diabetes and hypertension are interrelated as 75% of diabetic patients are known to be hypertensive (Lago, 2007; Bereketoglu, 2012) Type-2 diabetic patients are more prone to develop hypertension which is a major risk factor of cardiac, renal, and cerebral dysfunction (Golbidi *et al.*, 2012). Angiotensin-converting enzyme (ACE) converts angiotensin I to angiotensin II, a potent vasoconstrictor and stimulator of aldosterone secretion by the adrenal gland. Inhibition of angiotensin I-converting enzyme (ACE) is considered a useful therapeutic approach in the treatment of high blood pressure in both diabetic and nondiabetic patients (Crook & Penumalee, 2004; Johnston & Volhard, 1992). Recent studies indicating that phenolic compound rich - plants have the ability to inhibit ACE activity, both in vitro and in vivo (Actis-Goreta & Fraga, 2003; Kwon *et al.*, 2006). This opens up the possibility that medicinal plants that may mimic synthetic ACE inhibitors and provide health benefits, but without adverse side effects.

Since plants constitute a rich source of bioactive chemicals (Kador *et al.*, 1985a) and are largely free from adverse effects, they could possibly lead to the development of new classes of safer antidiabetic agents or diabetic complication resolving agents. Hence, the aim of this study was to screen some common Nigerian medicinal plants that could be useful in the management of diabetic complications. The plants and their pharmacological properties are highlighted below.

Morinda lucida is an evergreen tree or rarely a shrub 2.4–18 m tall, with smooth or rough scaly grey or brown bark and crooked or gnarled bole and branches. In Ghana and Nigeria, *Morinda lucida* is widely used in treating malaria, (Agbovie *et al*., 2002; Aiyeloja and Bello, 2006). According to Adomi (2006), the aqueous and ethanol bark extract of *M. lucida* were found to be potent against *S. aureus*, P. *aeruginosa*, *P. aeruginosa* and *E. coli*. Chukwujekwu (2005) also reported that ethanol extracts of *Morinda lucida* was active against *S. aureus.*

Alchornea cordifolia commonly known as Christmas bush is a small tree particularly native to tropical Africa. It is a shrubby tree that reaches 8-10 m in height with light-brown bark and violet flowers. It is widely distributed throughout Africa where it is used extensively in traditional medicine. It possesses potent antibacterial activity and could be beneficial in the management of different inflammatory disease states It has been very valuable locally in some ethnic groups in Nigeria for the management of haemorrhoids and high blood pressure and for its analgesic properties (Cesario, 1993).

Anthocleista vogelli known as the Cabbage tree is Native mainly of tropical Africa, Madagascar and Mascarene Islands; this species tends to occur in wet forest and has been recorded from Sierra Leone, Liberia and Nigeria. They are usually small trees or scrambling shrubs with soft white wood. Their leaves are glabrous, leathery and large and are often over one foot long in mature trees and up to 5 ft long in saplings. The leaves and stem-bark are used for treating swellings in the body (anti-inflammatory). The root-bark and leaves are used in local medicine (Dalzel, 1937).

Cassia sieberiana is a common tree in Nigeria. It is also found in East Africa. Previous studies showed that ethanolic root extract of *C. sieberiana* had an antiparasitic effect, myorelaxant and antispasmodic activity (Fall *et al.*, 2005). It was also shown that *C. sieberiana* extracts had antimicrobial activity against *Neisseria gonorrhoeae, Herpes simplex virus* type I and African swine fever virus (Silva *et al.*, 1997). In Senegal, the aqueous root extract of *C. sieberiana* was used in traditional medicine to treat pain and inflammation (Kerharo-Adam, 1974).

Nauclea latifolia commonly known as Pin cushion tree is a straggling shrub or small tree native to tropical Africa and Asia. It is a tropical plant that grows commonly in most parts of the Nigeria (Akpanabiatu *et al.,* 2005; Gidado *et al.,* 2009). It is found in the forest and fringe tropical forest. Its medicinal uses includes as tonic and for the treatment of fever medicine, toothaches, dental caries, septic mouth and malaria, diarrhoea and dysentery (Lamidi *et al.*, 1995). Parts of the plant are commonly prescribed traditionally as a remedy for diabetes mellitus. The plant is also used in the treatment of ailments such as malaria (Kokwaro, 1976; Boye, 1990), gastrointestinal tract disorders (Maduabunyi, 1995), sleeping sickness (Kerharo, 1974), prolong menstrual flow (Elujoba, 1995), hypertension (Akabue and Mittal, 1982) and as a chewing stick (Asubiojo *et al.,* 1982).

2. Materials and Methods

2.1 Materials

Bz- Glycyl-histidyl-leucine (GHL), Gallic acid, Folin-Ciocalticeau phenol reagent and 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH), yeast α-glucosidase enzyme, p-nitrophenyl-α-D-glucopyranoside, acarbose and trichloroacetic acid (TCA) were purchased from Sigma Chemical Co. Ltd (USA). All other chemicals and solvents used in this study were of analytical grade and were acquired from BDH, Poole, England.

2.2 Methods

2.2.1 Plant Collection and Identification

The leaves of *Morinda lucida, Alchornea cordifolia, Anthocleista vogelli, Cassia sieberena* and *Nauclea latifolia* were bought from an herbal market at Ajaka, Igalamela/ Odolu Local Government Area, Kogi State, Nigeria. The plants were identified at the herbarium unit of Biological Science Department, Federal University, Lokoja and voucher specimens were deposited for future references.

2.2.2 Preparation of Extracts

The leaves of *Morinda lucida, Alchornea cordifolia, Anthocleista vogelli, Cassia sieberena* and *Nauclea latifolia* were shade- dried for seven (7) days and pulverized separately using an electric blender. Five hundred (500) gram of the pulverized leaves of each plant was cold- macerated in methanol for 48- hours. The mixtures were subsequently filtered using Whatmann filter paper (Size No1) and the extracts were concentrated using a rotary evaporator. The extracts were weighed and percentage yield calculated for each extract.

2.2.3 Qualitative Phytochemical Analysis

Phytochemical analysis of the methanol extract of selected medicinal plants was carried out adopting the standard methods (Dhivya & Manimegalai, 2013) provided for the presence and absence of metabolites such as Alkaloids, Flavonoids, Phenol, Saponins, Steroids and Tannins was carried out.

2.2.4 Determination of Total Polyphenol Content

Total polyphenol content was determined using the Folin reagent as described by Ayoola and coworkers (Ayoola *et al.,* 2008). Concentrations of 12.5, 25, 50, and 100 µg/ml of gallic acid were prepared in methanol for preparation of standard calibration curve. Concentrations of 0.1g/ml of plant extracts were also prepared in methanol and 0.5 ml of each sample was mixed with 2.5 ml of a ten-fold diluted Folin-Ciocalteau's reagent and 2 ml of 7.5% sodium carbonate. The mixture was allowed to stand for 30 min at room temperature before the absorbance was read at 760nm using a spectrophotometer. All determinations were performed in triplicates, and the total phenolic content was expressed in terms of gallic acid equivalent (GAE).

2.2.5 Determination of Total Flavonoids Content

The total flavonoids content (TFC) was estimated spectrophotometrically by the aluminum chloride method based on the formation of flavonoids-aluminum complex as described by Pham *et al.,* (2007). The sample (1 ml) was mixed with 1 ml of $AlCl_3$ in methanol (2% w/v) and incubated at room temperature for 15 min. The absorbance was then read at 430 nm, and the amounts of TFC were estimated from the standard calibration curve of 12.5- 100 µg ml^{-1} quercetin, and hence, expressed in terms of quercetin equivalents (QE).

2.2.6 Determination of *in vitro* Antioxidant Activity

The scavenging activity of the extracts on 1, 1-diphenyl-2-picrylhydrazine (DPPH) was determined at 517nm using Trolox as standard following the procedure described by Atawodi *et al.,* (2010). Briefly, an aliquot (50 µl) of either the sample extract or standard Trolox solution was added to 2 ml of methanolic DPPH solution (0.1 mM), 0.95 ml of 0.05 M Tris- HCl buffer (pH 7.4) and wrapped in aluminum foil to reduce influence of light. The absorbance was measured at 517 nm exactly 30 seconds after adding each of the extracts. A loss of absorbance at this wavelength was taken as a measure of the radical scavenging capacity of the extract added. The antioxidant capacity based on the DPPH free radical scavenging ability of the extract was expressed in terms of Trolox equivalent (TE) after extrapolation from a Trolox standard calibration curve of 20 to 200 µg g^{-1}.

2.2.7 *In-vitro* α-Glucosidase Inhibition Assay

Different concentrations of the extracts (10- 100 μg/ ml) (50 μL) and 100 μL of α-glucosidase solution (1.0 U/ml) in 0.1M phosphate buffer (pH 6.9) were incubated at 25^{o}C for 10min. Then, 50 μL of 5mM p-nitrophenyl-α-D-glucopyranoside solution in 0.1M phosphate buffer (pH 6.9) was added. The mixtures were incubated at 25^{o}C for 5min, before reading the absorbance at 405 nm in the spectrophotometer. The α- glucosidase inhibitory activity was expressed as percentage inhibition (Apostolidis *et al.*, 2007).

2.2.8 *In-vitro* Aldose Reductase Inhibition (AR) Assay

Crude AR was prepared as in the following steps: lenses were removed from albino rats weighing 200-250 g, and were kept frozen until use. A homogenate of rat lens was prepared in accordance with the method described by Hayman and Kinoshita (1965). A partially purified enzyme, with a specific activity of 6.5 U/mg, was routinely used in the evaluations of enzyme inhibition. The partially purified material was separated into 1.0 ml aliquots, and stored at -40°C. The AR activity was spectrophotometrically assayed by measuring the decrease in NADPH absorption at 340 nm over a 4 min period, using L- glyceraldehydes as a substrate. Each 1.0 ml cuvette containing equal units of enzyme, 0.1M sodium phosphate buffer (pH 6.2) and 0.3 mM NADPH either with or without 10 mM substrate and inhibitor was prepared (Lim *et al.*, 2006). One set of mixtures prepared with an equivalent volume of sodium phosphate buffer instead of tested samples was used as control. The concentration of the extracts required to inhibit 50% of AR activity under the assay conditions was defined as the IC_{50} value.

2.2.9 *In-vitro* Angiotensin Converting Enzyme (ACE) Inhibition Assay

Different concentrations of the extracts (10- 100 μg/ ml) (50 μL) and ACE solution (50 μL, 4mU) were incubated at 37^{o}C for 15min. The enzymatic reaction was initiated by adding 150 μL of 8.33mM of the substrate Bz- Gly- His- Leu in 125mM Tris-HCl buffer (pH 8.3) to the mixture. After incubation for 30 min at 37^{o}C, the reaction was arrested by adding 250 μL of 1M HCl. The Gly- His bond was then cleaved and the Bz- Gly produced by the reaction was extracted with 1.5 ml ethyl acetate. Thereafter the mixture was centrifuged to separate the ethyl acetate layer; then 1 ml of the ethyl acetate layer was transferred to a clean test tube and evaporated. The residue was redissolved in distilled water and its absorbance was measured at 228 nm. The ACE inhibitory activity was expressed as percentage inhibition (Cushman and Cheung, 1971).

2.2.10 Statistical Analysis

The results of three replicates were pooled and expressed as Mean ± Standard Deviation (S.D) and the statistical differences between the means were determined by one way analysis of variance (ANOVA) which was followed by LSD. $P \leq 0.05$ was considered significant. The extract concentration causing 50% enzyme activities (IC_{50}) value was determined using non-linear regression analysis with Graph Pad Prism version 5.00 (Graph Pad Inc.).

3. Results

3.1 Percentage Yield of the Extracts

The yields in the methanol extracts of the medicinal plants are shown in Table 1. The yield percentages of the extracts in decreasing order were as follows: *Nauclea latifolia* (14.23%) > *Morinda lucida* (13.81%) > *Cassia sieberena* (12.75%) > *Alchornea Cordifolia* (9.33%) > *Anthocleista vogelli* (6.50%).

Table 1: Yield of Extracts from the Medicinal Plants

Plant	Methanol extract yield (% w/w) *
Morinda lucida	**13.81**
Alchornea Cordifolia	**9.33**
Anthocleista vogelli	**6.50**
Cassia sieberena	**12.75**
Nauclea latifolia	**14.23**

***** Dried weight basis

3.2 Phytochemical Composition of the Methanol Extracts of the Medicinal Plants

Preliminary phytochemical screening carried out on the methanolic extracts of the plants revealed the presence of Alkaloids, phenols flavonoids and steroids in all the plant extracts. Saponins were present in all the extracts except that of *Alchornea cordifolia* while tannins were found only in the extracts of *Alchornea cordifolia* and *Nauclea latifolia* (Table 2).

Table 2: Phytochemical Composition of the Extracts

	Phytochemicals					
Extract	Alkaloids	Phenols	Flavonoids	Saponins	Steroids	Tannins
Morinda lucida	+	+	+	+	+	-
Alchornea Cordifolia	+	+	+	-	+	+
Anthocleista vogelli	+	+	+	+	+	-
Cassia sieberena	+	+	+	+	+	+
Nauclea latifolia	+	+	+	+	+	-

Note: += Present, - = Absent

3.3 Total Polyphenol Content of the Extracts

Figure 1 shows the total polyphenol content of the extracts expressed as gallic acid equivalent. MEML had the highest (178 ±2.3 µg GAE /g) levels of total polyphenol content while MECS had the least (70 ±1.7 µg GAE /g) content. MEAC, MEAV and MENL had total polyphenol content of 158 ±2.2, 100 ±1.2 and 120 ±2.8 µg GAE /g respectively.

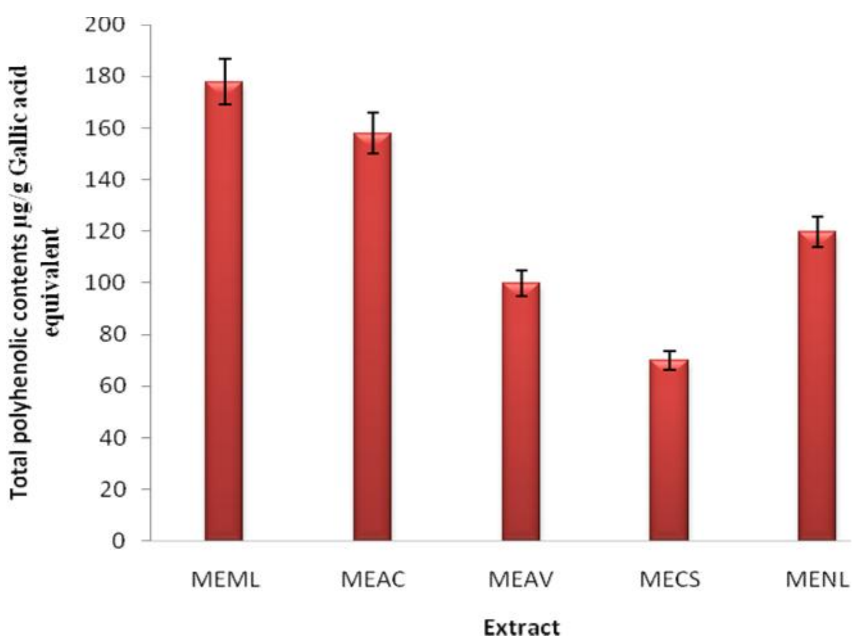

Figure 1: Total Polyphenol contents of the extracts
(Values presented are data from triplicate analyses expressed as Mean ± SD)
MEML= Methanol extract of *Morinda lucida leaves,* MEAC= Methanol extract of *Alchornea cordifolia* leaves, MEAV = Methanol extract of *Anthocleista vogelli* leaves, MECS= Methanol extract of *Cassia sieberena* leaves, MENL= Methanol extract of *Nauclea latifolia* leaves

3.4 Total Flavonoid Content of the Extracts

The total flavonoid content of the extracts expressed as quercetin equivalent is shown in Figure 2. Similar to the total polyphenolic content, MEML had the highest (48 ±0.9 µg QE /g) levels of total flavonoid content while MECS had the least (26 ±0.5 µg QE /g) content. MEAC, MEAV and MENL had total polyphenol content of 40 ±1.2, 33 ±0.8 and 38 ±0.8 µg QE /g respectively.

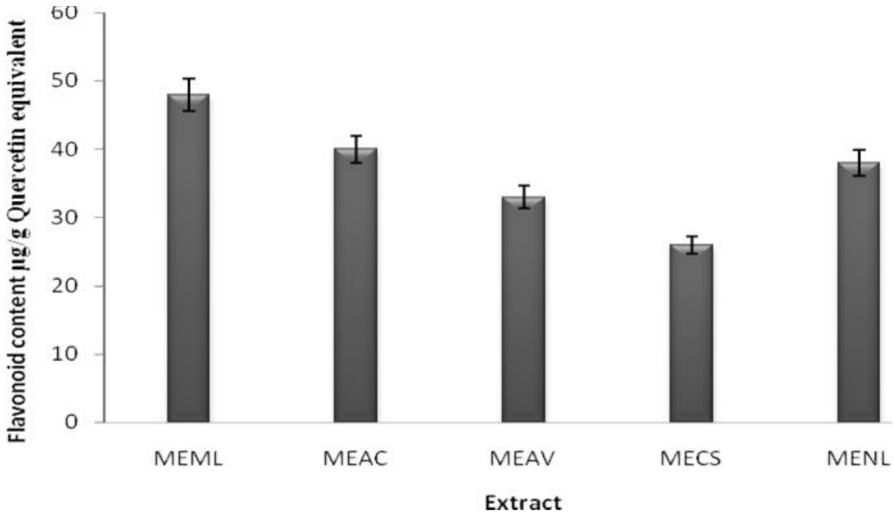

Figure 2: Total Flavonoid contents of the extracts
(Values presented are data from triplicate analyses expressed as Mean ± SD)
MEML= Methanol extract of *Morinda lucida leaves,* MEAC= Methanol extract of *Alchornea cordifolia* leaves, MEAV = Methanol extract of *Anthocleista vogelli* leaves, MECS= Methanol extract of *Cassia sieberena* leaves, MENL= Methanol extract of *Nauclea latifolia* leaves

3.5 DPPH Free Radical Scavenging Activity of the Extracts

The *in vitro* antioxidant activity of the plants showed similar trends with that of the total polyphenol and the total flavonoids. The total antioxidant activity of the extracts expressed as Trolox equivalent is shown in Figure 3. MEML showed the highest antioxidant activity with a value of 185±3.2 µg TE/g. MEAC, MEAV, MECS and MENL had values of 150 ±1.3, 79 ±0.6, 71 ±0.9, and 87±1.1 µg TE/g respectively.

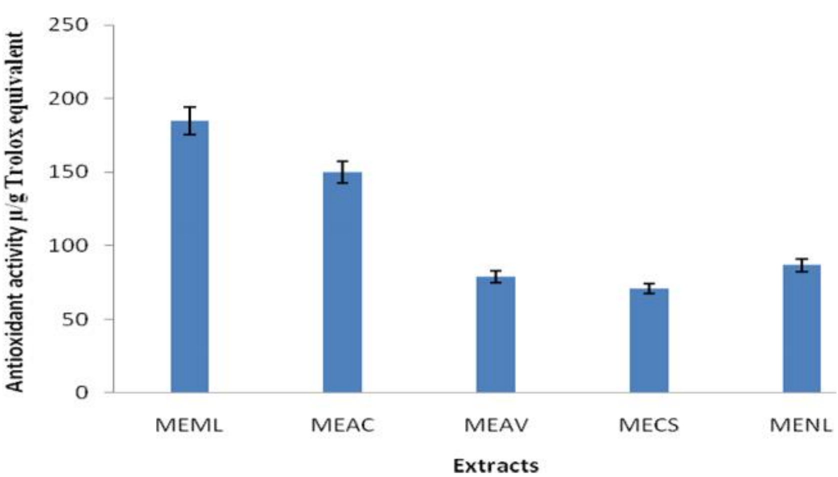

Figure 3: DPPH free radical scavenging activity of the extracts
(Values presented are data from triplicate analyses expressed as Mean ± SD)
MEML= Methanol extract of *Morinda lucida leaves,* MEAC= Methanol extract of *Alchornea cordifolia* leaves, MEAV = Methanol extract of *Anthocleista vogelli* leaves, MECS= Methanol extract of *Cassia sieberena* leaves, MENL= Methanol extract of *Nauclea latifolia* leaves

3.6 Correlation between Antioxidant Activity/ Total Polyphenol Content and Antioxidant Activity/ Total Flavonoid Content of the Extracts

The correlation analysis revealed a strong positive correlation between the antioxidant activity and the polyphenols contents (r^2 = 0.9028) and between the total flavonoids contents and antioxidant activity (r^2 = 0.8007) as shown in Figures 4 and 5 respectively.

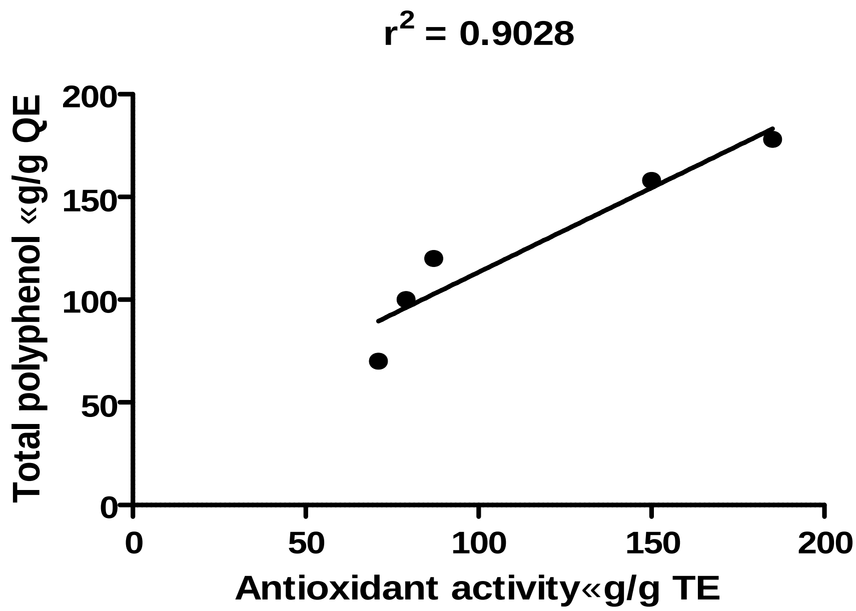

Figure 4: Correlation between antioxidant activity and total polyphenol content of the extracts

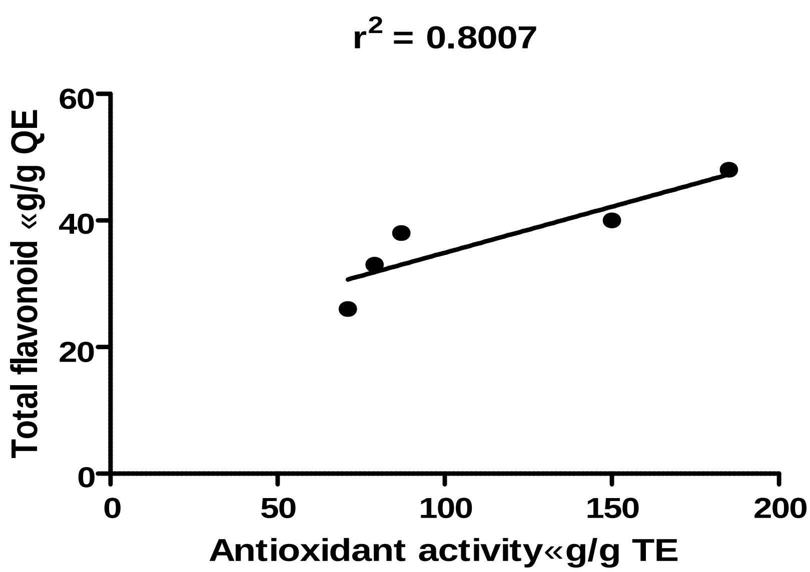

Figure 5: Correlation between antioxidant activity and total flavonoid content of the extracts

3.7 Inhibitory effect of the various concentrations of the Extracts on the Activity of α-Glucosidase

The extracts produced a concentration- dependent inhibitory effect on α- Glucosidase activity. MEML had the least median inhibitory concentration (IC_{50}) (49.95µg/ ml) while MEAV had the highest IC_{50} value (144.10 µg/ ml). The estimated values for MENL, MESC and MEAC were 61.36, 105.10 and 134.20 µg/ ml respectively. The standard drug, Acarbose had an IC_{50} value of 64.45µg/ ml.

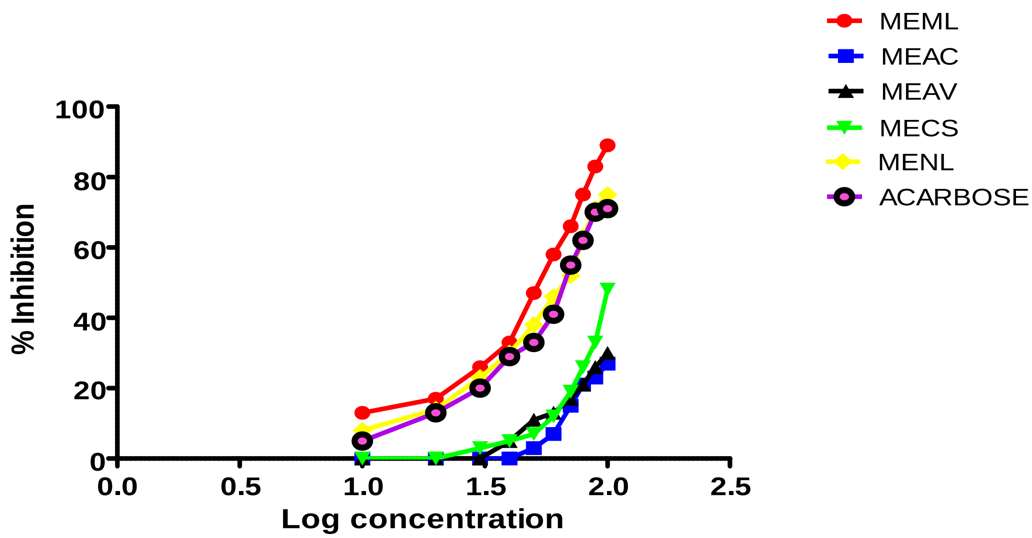

Figure 6: Concentration- Percentage Inhibition of α-glucosidase Curve for MEML, MEAC, MEAV, MECS, and MENL.
MEML= Methanol extract of *Morinda lucida leaves,* MEAC= Methanol extract of *Alchornea cordifolia* leaves, MEAV = Methanol extract of *Anthocleista vogelli* leaves, MECS= Methanol extract of *Cassia sieberena* leaves, MENL= Methanol extract of *Nauclea latifolia* leaves

3.8 Inhibitory Effect of the various Concentrations of the Extracts on the Activity of Aldose Reductase

The extracts also produced a concentration- dependent inhibitory effect on aldose reductase activity. MEAC had the least IC_{50} value (92.20 µg/ ml) while MECS had the highest value (162.10 µg/ ml). The IC_{50} of MEML, MEAV and MENL were estimated to be 137.30 and 130.50 µg/ ml respectively.

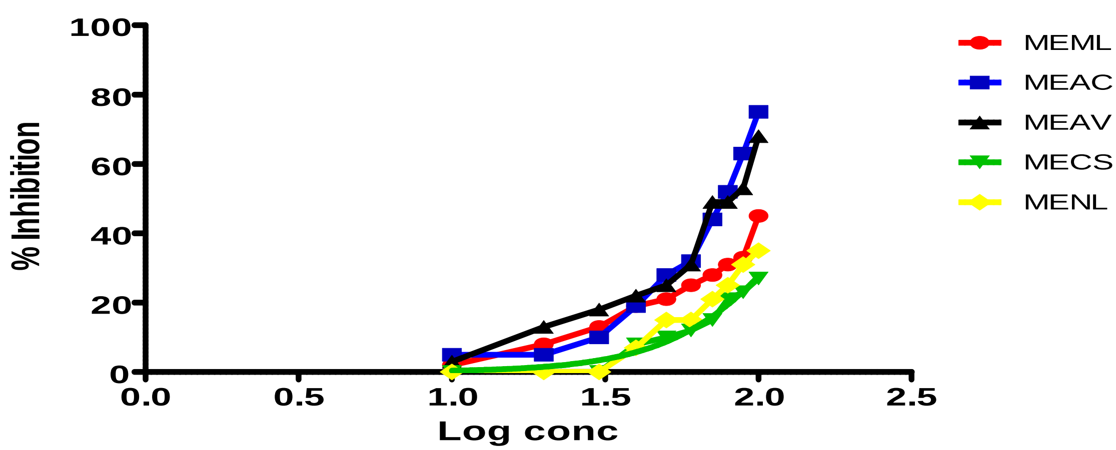

Figure 7:Concentration-Percentage Inhibition of Aldose Reductase Curve for MEML, MEAC, MEAV, MECS, and MENL.
MEML= Methanol extract of *Morinda lucida leaves,* MEAC= Methanol extract of *Alchornea cordifolia* leaves, MEAV = Methanol extract of *Anthocleista vogelli* leaves, MECS= Methanol extract of *Cassia sieberena* leaves, MENL= Methanol extract of *Nauclea latifolia* leaves

3.9 Inhibitory Effect of the Various Concentrations of the Extracts on the Activity of Angiotensin Converting Enzyme (ACE)

A concentration- dependent inhibitory effect on ACE activity was observed. MENL had the least IC_{50} value of 58.76 µg/ ml while MEAV had the highest value of 159.10 µg/ ml. MEML, MEAC and MECS had IC_{50} values of 69.47, 146.9 and 100.30 µg/ ml. The IC_{50} of the standard drug, Captopril was estimated to be 63.26 µg/ ml.

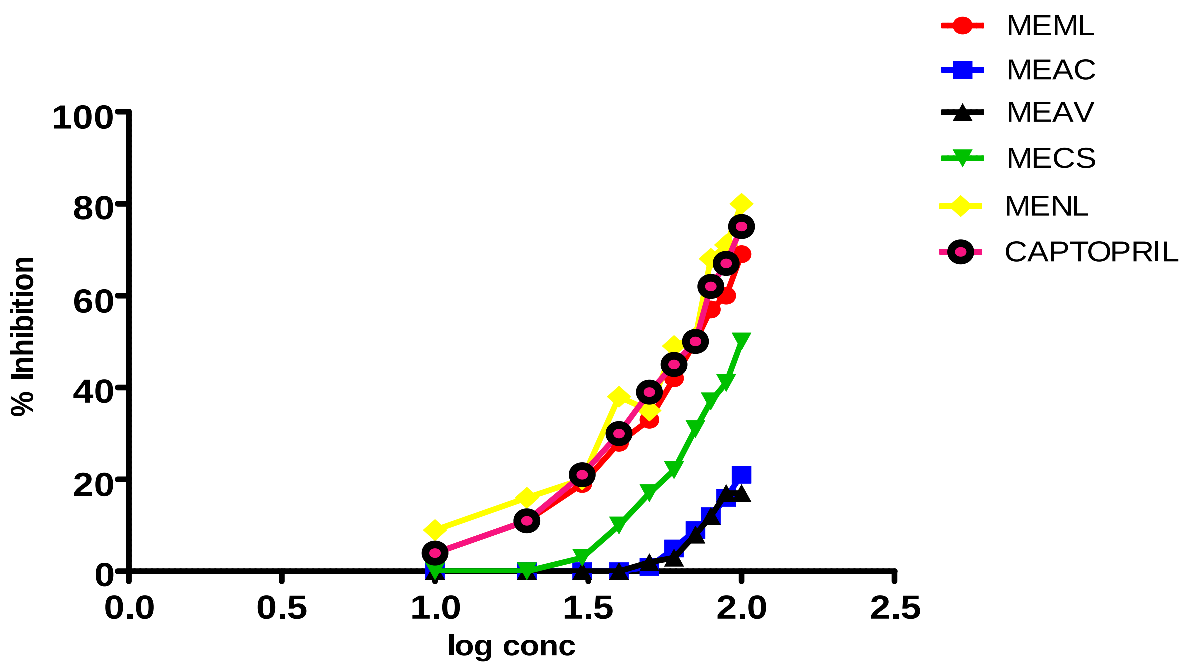

Figure 8: Concentration- Percentage Inhibition of ACE Curve for MEML, MEAC, MEAV, MECS, and MENL.
MEML= Methanol extract of *Morinda lucida leaves,* MEAC= Methanol extract of *Alchornea cordifolia* leaves, MEAV = Methanol extract of *Anthocleista vogelli* leaves, MECS= Methanol extract of *Cassia sieberena* leaves, MENL= Methanol extract of *Nauclea latifolia* leaves

4. 0 Discussion

The preliminary qualitative phytochemical analysis showed that the extracts of the plants contain alkaloids, phenols, flavonoids, steroids, saponins and tannins. Phytochemicals are known to exhibit physiological activity (Lachman *et al*., 1989). Phenolic acids and flavonoids are well known subclass of phytochemical principles with antioxidant properties and are used for the treatment of various ailments (Barnes, 2001). In this study, the methanol extract of *Morinda lucida* had the highest (178 ±2.3 µg GAE /g) estimated total polyphenol content while the extract of *Cassia sieberena* had the least (70 ±1.7 µg GAE /g) polyphenol content. Phenolic compounds exhibit antidiabetic properties through various mechanisms such as inhibition of carbohydrate digestion (by inhibiting alpha amylase and alpha glucosidase), glucose absorption in the intestine; stimulation of insulin secretion from pancreatic- β-cells, modulation of signaling pathways and gene expression among others (Bahadoran *et al*., 2013).

The total flavonoid content of the extracts followed the same trend as the total polyphenolic content, the extract of *Morinda lucida* had the highest (48 ±0.9 µg QE /g) total flavonoid content while the extract of *Cassia sieberena* had the least (26 ±0.5 µg QE /g) flavonoid content. Flavonoids, due to their redox abilities contribute to the total antioxidant activity. The mechanisms of the antioxidant activity of flavonoids in cells include neutralizing free radicals and preventing decomposition of hydroperoxides into free radicals that subsequently damage cells (Li *et al*., 2009) and hence, have potential in the management of diabetic complications (Kim *et al*., 2016).

The DPPH assay further confirms the antioxidant capacity of the aforementioned phytochemicals. DPPH assay is generally used method to evaluate the free radical scavenging power of medicinal plants. The DPPH radical involves a hydrogen atom transfer process (Kaviarasan *et al.,* 2007). The result of DPPH scavenging activity indicates that the extracts contain compounds that are capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for radical's reactivity. The methanol extract of *Morinda lucida* again showed the highest antioxidant activity with a value of 185±3.2 µg TE/g while *cassia sieberena* showed the least (71 ±0.9 µg TE/g) total antioxidant activity. To further establish the relationship between the total polyphenols, the flavonoids contents and the antioxidant activity, a correlation analysis carried out showed a strong positive correlation between the antioxidant activity and the polyphenols contents (r^2 = 0.9028) and between total flavonoids contents and *in vitro* antioxidant activity (r^2 = 0.8007). Increased levels of reactive oxygen species are seen in diabetic patient. Hyperglycemia coupled with oxidative stress favors glycation reactions and subsequently contributes to the diabetic complications and other degenerative diseases. This study demonstrated that the extracts particularly *Morinda lucida* possess relatively good radical scavenging property and hence could be useful in the prevention and management of diabetic complications.

A modern therapeutic approach to the management of diabetes and its related complications is the inhibition of starch metabolizing enzymes such as α-amylase and α- glucosidase (Shim *et al.,* 2003) as this will slow down the catabolism of starch into glucose and ultimately moderate the blood glucose level (Kwon *et al.,* 2007). As presented in this study, the extracts showed a concentration-dependent inhibition of α-glucosidase activity. It was observed that the extract of *Morinda lucida* had the least median inhibitory concentration (IC_{50}) (49.95µg/ ml), hence the most potent inhibitor of α- glucosidase activity. Interestingly, this IC_{50} value was significantly ($p< 0.05$) lower than that of the standard drug (Acarbose) used. Acarbose had an IC_{50} value of 64.45µg/ ml. This observation is therapeutically important, as the extract can be employed in the management of diabetes and its complication, thereby avoiding some of the side effects associated with the use of synthetic α-amylase and α-glucosidase inhibitors. Tannins and polyphenolic principles from plant extracts have shown significant inhibition of this enzyme (McDougall *et al.,* 2005). Therefore, the presence of tannins, alkaloids and flavonoids present in the plant extracts may be responsible for this observed activity.

Prolonged hyperglycemia leads to channeling of glucose into polyol pathway. Aldose reductase reduces glucose to sorbitol and then subsequently metabolized to fructose by sorbitol dehydrogenase. Normally this accounts for less than 3% of glucose consumption. However, in the presence of high glucose, the activity of this pathway is substantially increased and could represent up to 30% of total glucose consumption. Sorbitol changes the osmolar balance inside the cell leading to osmotic damage of the cell, and also use of NADPH for the pathway results in depletion of the NADPH thus resulting in oxidative stress (Giugliano *et al.,* 1996). Thus controlling flux through polyol pathway is a potential target for the control of sorbitol biogenesis. Aldose reductase is a rate limiting enzyme for the pathway hence aldose reductase inhibition can be potentially used to treat diabetic complication in the early stage. The extracts also produced a concentration- dependent inhibitory effect on aldose reductase activity. The extract of *Alchornea cordifolia* had the least IC_{50} value of 92.20 µg/ ml, hence the most potent inhibitor of aldose reductase activity. This value is significantly ($p< 0.05$) smaller compared to those of the other extracts. Plant phytochemicals particularly flavonoids and polyphenols as well as sugar derivatives are found to be effective in inhibiting aldose reductase enzyme (Hsieh *et al.,* 2010).

Similarly, the inhibition of Angiotensin- 1 converting enzyme (ACE) activity is a modern therapeutic approach in the management of hypertension which is one of the complications associated with type- 2 diabetes (Kwon *et al.,* 2006). ACE catalyses the conversion of angiotensin- I to angiotensin- II, a potent vasoconstrictor implicated in the elevation of blood pressure (Ahnfelt-Ronne, 1991). Therefore the inhibition of ACE is useful in the management of hypertension. The extracts inhibited ACE activity *in vitro* in a concentration- dependent manner. *Nauclea latifolia* with IC_{50} value of 58.76 µg/ ml showed the highest inhibitory action against ACE activity. The extract showed better inhibition than the standard drug, Captopril with an estimated IC_{50} of 63.26 µg/ ml. This extract therefore, could be employed as an alternative ACE inhibitor thereby avoiding some of the side effects associated with the use of synthetic ACE inhibitors.

5.0 Conclusion

All the extracts possess antioxidant activity as well as inhibitory action on α-glucosidase, aldose reductase and angiotensin converting enzyme activities. However, there were variations in the activities of the extracts. *Morinda lucida* had the highest antioxidant activity and it was the most potent inhibitor of α-glucosidase activity, *Alchornea cordifolia* was the most potent against the activity of aldose reductase while *Nauclea latifolia* was the most potent inhibitor of ACE activity. These plants individually or as a polyherbal formulation, could be useful in the management of diabetic complications. However, further investigations are recommended.

References

Actis-Goreta, L., Ottaviani, J. I., Keen, C. L., Fraga, G. 2003. Inhibition of angiotensin converting enzyme (ACE) activity by flavan-3-ols and procyanidins. FEBS Letters, 555, 597−600.

Adomi, P.O. 2006. Antibacterial activity of aqueous and ethanol extracts of the stem bark of Alstonia boonei and *Morinda lucida*. S*cientific Research and Essay*. 1 (2): 050-053

Agbovie, T. K; Amponsah, O. R; Cretsil, F; Dennis, G.T; Odamtten, W and Ofusohene-Djan D. 2002. Conservation and Sustainable use of medicinal plants in Ghana. *Ethnobotanical Survey*. Ed. F. Dennis. Available on www.unepwcmc.org/species/plants/-ghana

Ahnfelt-Ronne, "Enzyme inhibitors as drugs," in *A Textbook of Drug Design and Development*, P. Krogsgaard-Larsen and H. Bundgaard, Eds., pp. 302–307, Harvood Academic Publishers, Chur, Switzerland, 1991.

Aiyeloja, A. A, Bello, O. A. 2006. Typhoid Fever. Educational Research and Review (1): 16-22.

Akabue, P., Mittal, G. C. 1982. Clinical Evaluation of a Traditional Herbal Practice in Nigeria: a Preliminary Rep. *J of Ethnopharmacology*. 6(3):355- 359.

Akpanabiatu, M. I; Umoh, I. B; Udosen, E. O; Udoh, A. E., Edet, E. E. 2005. Rat serum electrolytes, lipid profile and cardiovascular Activity on *Nauclea latifolia* leaf extract administration. *Indian J of Clin Biochem*. 20 (2): 29-34.

Altan, V. M. The pharmacology of diabetic complications. 2003. *Curr. Med. Chem.*; 10: 1317–1327.

Andrew, J. K. 2000. Diabetes. Churchill living stone: New York.

Apostolidis, E., Kwon, Y. I., Shetty, K. 2007. "Inhibitory potential of herb, fruit, and fungal-enriched cheese against key enzymes linked to type 2 diabetes and hypertension," *Innov. Food Sci. Emerg. Technol*, 8; 1, 46–54.

Argoff, C. E., Cole, B. E., Fishbain, D. A., Irving, G. A. 2006. Diabetic peripheral neuropathic pain: Clinical and quality of life issues. *Mayo Clin Proc*; 81: S3- S6

Asubiojo, O.I; Guinn, V.P., Okunuga, A. 1982. Multi-element Analysis of Nigerian Chewing Sticks by Instrumental Neuron Activation Analysis. *J of Radio Anal. Chem.* 74: 149 -156.

Atawodi, S. E, Atawodi, J. C, Idakwo, G. A, Pfundstein, B, Hambner R, Wutela G, Bartsch H, Owen RW. 2010. Evaluation of the polyphenols contents and antioxidants properties of methanol extracts of leaves, stem and root barks of *Moringa oleifera* Lam. *J Med Food*; 13(3):710.

Ayoola, G. A, Folawewo, A. D, Adesegun, S. A, Abioro, O. O, Adepoju-Bello, A. A, Coker H.A.B. 2008. Phytochemical and antioxidant screening of some plants of apocynaceae from South West Nigeria. *Afri J Plant Sci;* 2(9):124. 4282

Bahadoran, Z.,Mirmiran, P., Azizi, F.2013.Dietary polyphenols as potential nutraceuticals in management of diabetes: a review. *J. Diabetes Metab.Disord*.12,1–9.doi:10.1186/2251-6581-12-43.

Barnes, S. 2001. Role of phytochemicals in prevention and treatment of prostate cancer. *Epidemiol. Rev*. 23(1):201–205.

Bereketoglu, C.; Kasap, M.; Pazarbas, A. Studies on Angiotensin-Converting Enzyme Insertion/Deletion Polymorphism and Genotype Distributions in Turkish Preeclampsia Patients. Journal of Pregnancy 2012. DOI:10.1155/2012/108206.

Boulton, A. J. M., Vinik, A. I., Arezzo, J. C., Bril, V., Feldman, E. L., Freeman, R., Malik, R. A., Maser, R. E., Sosenko, J. M.,Ziegler, D. 2005. Diabetic neuropathies: A statement by the American Diabetes Association. Diabetes Care; 28: 956-962.

Boulton, A. J. 2005. Management of diabetic peripheral neuropathy.Clinical Diabetes; 23: 9-15.

Boye, G. L. 1990. Studies on Antimalarial Action of *Cryptolepis sanguinolenta Extracts of Proceedings of International Symposium on East-West Medicine*. Seoul, Korea. p 243 – 251.

Cesario, A. 1993. Screening of plants used in Argentine folk medicine for antimicrobial activity. *Journal of Ethnopharmacology*. 139:119- 128.

Chukwujekwu, J. C; van Staden, J, Smith, P. 2005. Antibacterial, anti-inflammatory and antimalarial activities of some Nigerian medicinal plants. *South African Journal of Botany*. 71(3): 0254-0259

Crook, E. D. Penumalee, S. 2004. Therapeutic controversies in hypertension management: Angiotensin converting enzyme (ACE) inhibitors or angiotensin receptor blockers in diabetic nephropathy? ACE inhibitors. Ethnicity and Disease, 14, S2-1-4.

Cushman, D. W, Cheung, H. S "Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung," *Biochemical Pharmacology*, vol. 20, no. 7, pp. 1637–1648, 1971.

Dalziel J.M. 1937. The useful plants of West Africa. Crown Agents, London.

DeFronzo, R.A. Pharmacologic therapy for type 2 diabetes mellitus. Annals of Internal Medicine 1999; 131: 281–303.

Dhivya R, Manimegalai K. Preliminary Phytochemical Screening and GC- MS Profiling of Ethanolic Flower Extract of Calotropis gigantea Linn. (Apocynaceae).2013. Journal of Pharmacognosy and Phytochemistry; 2(3):28-32.

Elujoba, A. A. A. 1995. Female Infertility in the Hands of Traditional Birth Attendants in South-West Nigeria. *Fitoterapia* 66(3): 239 – 248.

Fall, A.D; Diatta, W; Sy, G.Y; Lo, M; Bassène, E. and Faye, B. 2005. Activité myorelaxante et antispasmodique des fractions de l'extrait total éthanolique de racines de *Cassia sieberiana* D.C. (*Caesalpiniaceae*) sur l'intestin isolé de rat. Dakar Méd. 50(3): 132-135.

Fowler MJ. Microvascular and macrovascular complications of diabetes. Clin Diab 2008; 26:77–82

Fred-Jaiyesimi, A., Kio, A. and Richard, W. 2009. Alpha-Amylase inhibitory effect of 3β-olean-12-en-3-yl (9Z)-hexadec-9-enoate isolated from *Spondias mombin* leaf. *Food Chem;* 116: 285-288.

Gidado, A; Ameh, D.A; Atawodi, S.E. and Ibrahim, S. 2009. Antidiabetic Effect of *Nauclea latifolia* Leaf Ethanolic Extract in Streptozotocin-induced Diabetic Rats. *Pharmacognosy Research*. 6(1): 392-395

Giugliano D, Ceriello A, Paolisso G. 1996. Oxidative stress and diabetic vascular complications. *Diabetes care, Mar;* 19(3): 257-67.

Golbidi, S., Ebadi, S.A.; Laher, 2011. I. Antioxidants in the Treatment of Diabetes. *Current Diabetes Review*, 7 (2), 106–125.

Guzmán 1A, Guerrero RO. Inhibition of aldose reductase by herbs extracts and natural substances and their role in prevention of cataracts. 2005. *J Rev Cubana Plant Med*. ;10:3-4.

Halder, N., Joshi, S., Gupta, S.K.Lens aldose reductase inhibiting potential of some indigenous *plants*. 2003. *J Ethnopharmacol*; 86:113–6.

Hall, G. C., Carroll, D., Parry, D. McQuay, H. J. 2006. Epidemiology and treatment of neuropathic pain: The UK primary care perspective. *Pain*; 122: 156-162.

Haraguchi, H., I. Ohmi, S. Sakai, and A. Fukuda. 1996. Effect of *Polygonum hydropiper* sulfated flavonoids on lens aldose reductase and related enzymes. *J. Nat. Prod*. 59**:** 443-445.

Hayman, S. and J.H. Kinoshita. 1965. Isolation and properties of lens aldose reductase. *J. Biol. Chem*. 240: 877-882.

Hsieh P, Huang G, Ho Y, Lin Y, Huang S, Chiang Y. 2010. Activities of antioxidants, alpha-glucosidase inhibitors and aldose reductase inhibitors of the aqueous extracts four Flemingia species in Taiwan. *Bot Stud*; 51: 293-302.

Iwai, K. 2008. Antidiabetic and antioxidant effects of polyphenols in brown algae *Ecklonia stolonifera* in genetically diabetic KK-A(y) mice. *Plant Foods Hum. Nutr.*, 63: 163-169.

Jin, L., Xue, H.Y., Jin, L.J., Li, S.Y. Xu, Y.P. 2008. Antioxidant and pancreas protective effect of aucubin on rats with streptozotocin-induced diabetes. *Eur. J. Pharmacol.*, 582: 162-167.

Johnston, J. -I., & Volhard, F. 1992. Renin–angiotensin system: A dual tissue and hormonal system for cardiovascular control. *Journal of Hypertension*, 10, 13−26.

Kador, P.F., W.G. Robison, and J.H. Kinoshita. 1985a. The pharmacology of aldose reductase inhibitors. *Annu. Rev. Pharmacol. Toxicol*. 25: 691-714.

Kador, P.J., J.H. Konishita, and N.E. Sharpless. 1985b. Aldose reductase inhibitors: a potential new class of agents for the pharmacological control of certain diabetic complications. J. Med. Chem. 28: 841-849.

Kaviarasan, S., Naik, G.H., Gangabhagirathi, R., Anuradha, C.V. and Priyadarsini, K.I. 2007. In vitro studies on antiradical and antioxidant activities of fenugreek (*Trigonella foenumgraecum*) seeds. *Food Chem.*103, 31–37.

Kerharo-Adam, J.G, 1974. Pharmacopée sénégalaise traditionnelle, plantes médicinales et toxiques. Vigot et Frères; *Paris*. p. 1011.

Kim,Y., Keogh, J.B., and Clifton, P. M. 2016.Polyphenols and glycemic control. *Nutrients* 8:17.

Kokwaro, J.O. 1976. Medicinal Plants of East Africa. East African Literature Bureau, Nairobi.

Kwon, Y. I, Apostolidis, E.,Kim, Y.C., Shetty, K.2007. "Health benefits of traditional corn, beans, and pumpkin: in vitro studies for hyperglycemia and hypertension management," *Journal of Medicinal Food*, vol. 10, no. 2, pp. 266–275.

Kwon, Y.I., Vattem, D.A. and Shetty K. 2006. "Evaluation of clonal herbs of Lamiaceae species for management of diabetes and hypertension," *Asia Pacific Journal of Clinical Nutrition*, vol. 15, no. 1, pp. 107–118.

Lachman, L., Lieberman, H.A. and Kanig, J. 1989. *The Theory and Practice of Industrial pharmacy, Edn 3*, Varghese publishing house, New York; 293-373.

Lago, R.M.; Singh, P.P.; Nesto, R.W. Diabetes and Hypertension. 2007. Nature Clinical Practice Endocrinology and Metabolism, 3, 667.

Lamidi, M; Ollivier, E; Faure, R; Debrauwer, L; Nze-Ekekang, L. and Balansard, G. 1995. Quinovic acid glycosides from *Nauclea diderichii*. *Planta Medicine* 61: 280-281.

Lee, H.S. and M.K. Kim. 2001. Rat intestinal R-glucosidase and lens aldose reductase inhibitory activities of grain extracts. *Food Sci. Biotechnol*. 10: 172-177.

Li H., Hao Z., Wang X., Huang L., Li J. 2009. Antioxidant activities of extracts and fractions from *Lysimachia foenum-graecum* Hance. *Bioresour. Technol*. 100:970–974.

Lim, S.S., Y.J. Jung, S.K. Hyun, Y.S. Lee, , J.S. Choi. 2006. Rat lens aldose reductase inhibitory constituents of *Nelumbo nucifera* stamens. *Phytother. Res.* 20: 825-830.

MacIsaac R, Jerums G. Management of early Diabetic Nephropathy.2003. *Diabetes Voice*; 48 (Special Issue):15–8.

Madubunyi, I.I. 1995. Anti- Hepatotoxic and Trypanocidal Activities of the Ethanolic Extract of *Nauclea latifolia* Root Bark. *J Herbs Spices Medicinal Plants*. 3 (2):23 – 53.

McDougall, G.J. and D. Stewart. 2005. The inhibitory effects of berry polyphenols on digestive enzymes. *Biofactors* 23: 189-195.

Merlin, T., Cori, T., Richard,M., George, J.Anaemia in Diabetes. 2995. An Emerging Complication of Microvascular Disease. *Curr Diab Rev*; 1:107-26.

Patel, D. K, Kumar, R, Kumar M, Sairam, K., Hemalatha, S. Evaluation of in vitro aldose reductase inhibitory potential of different fraction of *Hybanthus enneaspermus* Linn F. Muell. 2012. *Asian Pacific Journal of Tropical Biomedicine;* 18:134-9

Pham, T. Q, Tong, V. H, Nguyen. H. H, Bach LG. 2007. Total polyphenols, total catechins content and DPPH free radical scavenger activity of several types of Vietnam commercial green tea. *J Sci Technol Devt.;*10 (10):5-11.

Shim,Y. J, Doo, H.K, Ahn S.Y., "Inhibitory effect of aqueous extract from the gall of *Rhus chinensis* on alphaglucosidase activity and postprandial blood glucose," *J of Ethnopharm*, 85; 23, 283–287.

Silva, O; Barboza, S; Diniz, A; Valdeira, L. and Gomes, E. 1997. Plant extracts antiviral activity against Herpes simplex virus type I and African swinefever virus. *Int J of Pharm.* 35(1): 12-16.

Vos, T., Flaxman, A. D., Naghavi, M., Lozano, R., Michaud, C., Ezzati,M. 2012. Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990-2010: a systematic analysis for the global burden of disease study 2010. *Lancet* 380, 2163–2196.

World Health Organization. Fact sheet N° 282. 2009. Available from URL: http://www.who.int/ mediacentre/factsheets/ fs282/en/index.html

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