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AVOCADO PEAR FRUITS AND LEAVES AQUEOUS EXTRACTS INHIBIT α-AMYLASE, α-GLUCOSIDASE AND SNP INDUCED LIPID PEROXIDATION – AN INSIGHT INTO MECHANISMS INVOLVE IN MANAGEMENT OF TYPE 2 DIABETES

ADELUSI TEMITOPE ISAAC[1], OBOH GANIYU[2], AKINYEMI AYODELE JACOBSON[3], AJANI RICHARD AKINLOLU[4] & BAKARA OLALEKAN OLANREWAJU[5]

[1,4]Department of Biochemistry, Ladoke Akintola University of Technology Ogbomoso, Nigeria

[2,5]Functional Foods and Nutraceuticals Unit, Department of Biochemistry, Federal University of Technology, Akure, Nigeria

[3]Department of Biochemistry, Afe Babalola University, Ado-Ekiti, Nigeria

ABSTRACT

Introduction

The use of natural products for the management of diseases had been established in folk medicine. Avocado pear (*Persea americana*) is used in traditional medicine to manage type 2 diabetes mellitus. Therefore, the focus of this study was to investigate the mechanism behind its antidiabetic prowess by accessing the inhibitory activities of aqueous extract of leaves and fruit parts of avocado on α-amylase, α-glucosidase and malondialdehyde (MDA) produced by sodium nitropruside-induced lipid peroxidation in rats' pancreas *in vitro*.

Methods

The inhibitory effect was assessed using 5mg/ml aqueous extracts on α-amylase and α-glucosidase activities, ABTS (2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid)) radical, $NO^{\cdot}$ radical scavenging abilities and SNP-induced malondialdehyde produced after which the types and quantity of phenolics in the leaves and fruit parts of *Persea americana* were characterized.

Results

The leaves, peel, flesh and seed extracts inhibited α-amylase, α-glucosidase and the production of malondialdehyde in a dose dependent pattern. The minimum extract concentration that will inhibit 50% enzyme activity (IC_{50}) revealed that the peel showed the highest significant ($P < 0.05$) α-amylase and α-glucosidase inhibitory activities while the seed revealed the highest MDA inhibition, $NO^{\cdot}$ and ABTS radical scavenging abilities. Syringic acid, eugenol, vnillic acid, isoeugenol, guaiacol, phenol, kaempherol, catechin, ρ-hydroxybenzoic acid, ferulic acid, apigenin, naringenin, epigallocatechin, lupeol and epigallocatechin-3-O-gallate were revealed when the aqueous extracts of avocado pear leaf and fruit parts was characterized.

Conclusions

This work unravel the possible mechanisms (inhibition of α-amylase and α-glucosidase) used by avocado pear leaves and fruit parts to manage/treat diabetes type 2 and the bioactive phenolics that may take part in the process.

KEYWORDS: Type 2 diabetes, Malondialdehyde, *Persea americana*, α-amylase, α-glucosidase

INTRODUCTION

In type 2 diabetic patients, sudden rise in blood glucose level can cause hyperglycemia through the hydrolysis of starch and uptake of glucose by pancreatic α-amylase and intestinal α-glucosidase respectively (Kwon *et al*., 2007). Pancreatic α-amylase hydrolyzes complex starches to oligosaccharides in the lumen of the small intestine while intestinal α-glucosidase converts the oligosaccharides to monosaccharaides leading to hyperglycemia (Stewart, 2007). Postprandial hyperglycemia is a sudden rise in blood glucose level after meal. One of the therapeutic approaches for treating diabetes is to decrease postprandial hyperglycemia – a complication strictly linked to diabetes mellitus. The control/management of this sudden rise in blood glucose level can be achieved by delaying the absorption of glucose through the inhibition of carbohydrate hydrolyzing enzymes which are key enzymes linked to type 2 diabetes mellitus (pancreatic α-amylase and intestinal α-glucosidase) in the digestive tract (Lebovitz *et al*., 1997).

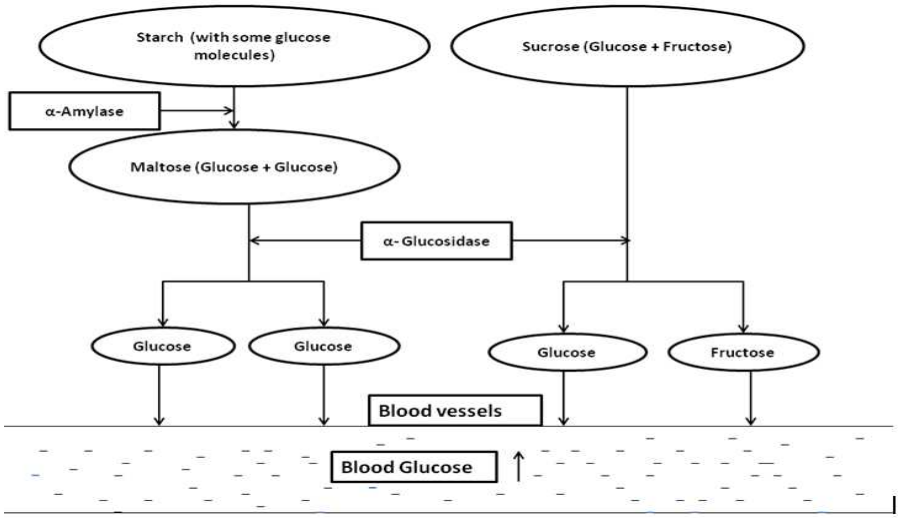

Figure 1: Schematic Diagram Illustrating the Effect of α-amylase and α-glucosidase on Digestion of Carbohydrate

The beneficial effects of several established enzyme inhibitors like acarbose, miglitol, voglibose, nojirimycin and 1-deoxynojirimycin on blood glucose levels after meal have been reported (Kim *et al*., 2005). Amylase inhibitors are called starch blockers because of their ability to prevent the digestion and absorption of starches into the body. Nutritional evaluations of some plants have shown storage of biologically active substances which include α-amylase inhibitors (McEwan, 2008). α-Amylase inhibitor proteins A-1 and B-2 were extracted and partially purified from *Colocasia esculenta* (McEwan *et al*., 2010). Medicinal plants had been shown to have potent α-glucosidase inhibitors as their bioactive ingredients. Thai medicinal plant is an example of a plant that possesses α-glucosidase inhibitors with health benefits. The inhibitory properties of 24 traditional Thai medicinal aqueous plant extracts on α-glucosidase had been examined. Devil tree leaf (*Alstonia scholaris*) extract exhibited strong inhibition against enzyme activities (Nulibon *et al*., 2007). Studies on the α-glucosidase inhibitors present in natural sources, such as plants, foodstuffs and microbes had been intensive for the past forty years (Fujita & Yamagami, 2001; Fujita *et al*., 2001). The mechanism of action of α-glucosidase inhibitors is to retard the liberation of glucose from dietary complex carbohydrates and therefore delay glucose absorption, resulting in reduced postprandial plasma glucose levels and suppress postprandial hyperglycemia (Puls *et al*., 1977; Lebovitz *et al*., 1997;). In drug design, α-amylase and α-glucosidase inhibitors have been the main targets used to develop compounds for the management of diabetes (Franco *et al*., 2002). Furthermore, other benefits of α-glucosidase inhibitors,

such as reducing triglycerides (Lebovitz, 1998), postprandial insulin levels (Johnston *et al*., 1994) and anti-HIV activity (Fischer *et al*., 1996a & b; Fujita & Yamagami, 2001; Fujita *et al*., 2001) had also been reported. Therefore, the discovery of plant preparations containing glucosidase inhibitors devoid of side effects present in oral anti-diabetic drugs for the management/prevention of type 2 diabetes have made researches for the natural anti-diabetic agents attractive.

Sodium nitropruside - an antihypertensive agent can also generate NO radical and therefore can be used to induce malondialdehyde (MDA) production in tissues (Salvemini *et al*., 1996; Moncada, 1997; Hariawala *et al*., 1997; Yahamoto *et al*., 2000). The NO released by this cytotoxic pro-oxidant had been shown to be involved in degenerative diseases (e.g. seizure disorders, trauma and stroke) (Oboh & Rocha, 2008). Increment in the body's antioxidant status through higher consumption of vegetables and fruits had also been proved by researchers to scavenge these free radicals (Ren-You *et al*., 2010).

Recently, scientists have diverted their interest into diabetes research making use of medicinal plants. Natural enzyme inhibitors from plant sources have offered an attractive strategy for the control of postprandial hyperglycemia (Onal *et al*., 2005). The interaction between proteins and the polyphenolic compounds present in these natural plants give them the access to inhibit enzymatic reactions (Dawra *et al*., 1988; Suryanarayana *et al*., 2004). Plant foods majorly compose of phenolic compounds which are antioxidants (Rice-Evans *et al*., 1996). Phenols are well-known for their cellular protective role against reactive oxygen species produced in energy metabolism (Passamonti *et al*., 2005). Antioxidants protect by reducing reaction (donation of electron or hydrogen atom), thereby neutralizing and stabilizing free radicals and help prevent against their deleterious effects to body cells and tissues (Balasundram *et al*., 2006). It has been established that the antioxidant prowess of phenols is strictly dependent on the relationship between different parts of their chemical structure (Rice-Evans *et al*., 1996). However, the inhibitory potential of several vegetables and herbal extracts on α-amylase and α-glucosidase qualify them to be among the class of dietary antidiabetic agents for the control of sudden rise in blood glucose level after meal (McCue *et al*., 2004).

Avocado pear serves as a good source of vitamin A, B, C, E, potassium (higher than banana) and fibers; fair source of iron and low in calcium. 65% of its high fat content is health-promoting monounsaturated, particularly oleic acid. Protein content of avocado fruit averages 2% (Owolabi *et al*., 2010). In ethno-medicine, plants have been well-known for their antidiabetic potential for many years (Ali *et al*., 2006). Besides the various antioxidant composition of avocado (*Persea americana*), it has been discovered that they also possess antidiabetic (Antia *et al*., 2005), hypolipidemic (Brai & Odetola, 2006), antiobesity (Brai *et al*., 2007) and hypotensive (Adeboye *et al*., 1999) prowess. Although there had been some reports on the nutritional importance and antioxidant properties of avocado pear, there is limited information on its ability to manage or control diabetes.

Therefore this study was meant to investigate the inhibitory effect of Avocado pear (*Persea americana)* aqueous leaf and fruit parts extracts on free radicals, key-enzymes linked to type-2 diabetes (α-amylase and α-glucosidase) and malondialdehyde produced by sodium nitroprusside induced lipid peroxidation in order to suggest the possible mechanisms behind its antidiabetic strength.

MATERIALS AND METHODS

Materials

The Avocado pear (*Persea americana)* leaves and fruits were obtained from a farm land at Ijoka, Akure, Ondo state. The authentication was carried out at the Department of Crop, Soil and Pest management, Federal University of

Technology, Akure, Nigeria. All chemicals and reagents used in this study were of analytical grade and glass-distilled water was used. Absorbance was measured using JENWAY UV-visible spectrophotometer (Model 6305; Jenway, Barlo world Scientific, Dunmow, United Kingdom).

Preparation of Aqueous Extracts

After the washing of the leaves and the fruit of avocado with distilled water to remove contaminants, the fruits were separated into three parts (peel, flesh and seed), chopped into small pieces and sundried. The aqueous extracts of these leaves and three fruit parts (peel, flesh and seed) were subsequently prepared by soaking the grinded samples in distilled water for 24hrs at 37^0C; mixtures were filtered, and the filtrates were stored in the refrigerator for subsequent analysis.

Lipid Peroxidation Assay

Preparation of Tissue Homogenates

The rats were sacrificed under mild diethyl ether anaesthesia and the pancreas was rapidly isolated, placed on ice and weighed. This tissue was subsequently homogenized in cold saline (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 a low-speed supernatant (SI) was kept for lipid peroxidation assay (Belle *et al*., 2004).

Lipid Peroxidation and Thiobarbituric Acid Reactions

The lipid peroxidation assay was carried out using the modified method of Ohkawa *et al*., (1979). Briefly 100µl of SI fraction was mixed with a reaction mixture containing 30µl of 0.1M pH 7.4 Tris-HCl buffer, extract (0 – 100 µl) and 30µl of 250µM freshly prepared $FeSO_4$ (the procedure was also carried out using 5µM sodium nitroprusside). The volume was made up to 300µl by water before incubation at 37^oC for 3hours. The colour reaction was developed by adding 300µl 8.1% SDS (Sodium deodecyl sulphate) to the reaction mixture containing SI; this was subsequently followed by the addition of 600µl of acetic acid/HCl (pH 3.4) mixture and 600µl 0.8% TBA (Thiobarbituric acid). This mixture was incubated at 100^oC for 1hour. TBARS (Thiobarbituric acid reactive species) produced were measured at 532 nm and expressed as Malondialdehyde (MDA) equivalent.

Enzyme Inhibition Assays

α- amylase Inhibition Assay

The α-amylase inhibitory activity was determined according to the method of Bernfield (1951). The aqueous extract (500µL) and 500 µL of 0.02 mol/l sodium phosphate buffer (pH 6.9 with 0.006mol/L NaCl) containing Hog pancreatic α – amylase (EC 3.2.1.1) (0.5 mg/ml) were incubated at 25^0C for 10 minutes. Then, 500 µL of 1% starch solution in 0.02mol/l sodium phosphate buffer (pH 6.9 with 0.006 mol/l NaCl) was added to the reacting mixture. Thereafter, the reaction mixture was then incubated in a boiling water bath for 5 minutes, and cooled to room temperature. The reaction mixture was then diluted by adding 10ml of distilled water, and absorbance measured at 540nm in the JENWAY UV-Visible spectrophotometer. The α-amylase inhibitory activity was expressed as percentage inhibition.

α- glucosidase Inhibition Assay

The α-Glucosidase inhibitory activity was determined according to the method of Apostolids *et al*. (2007). Appropriate dilution of the extract (50µL) and 100µL of α- glucosidase solution was incubated at 25^0C for 10 minutes.

Thereafter, 50 µL of 5 mmol/l *p*-nitrophenyl-α-D-glucopyranoside (Pnp) solutions in 0.1 mol/phosphate buffer (pH 6.9) was added. The reacting mixture was then incubated at 25^0C for 5 minutes, before reading the absorbance at 400nm in the JENWAY UV-Visible spectrophotometer. The α-glucosidase inhibitory activity was expressed as percentage inhibition.

2, 2'-Azino-bis (3-Ethylbenzthiazoline-6-Sulphonic acid) ($ABTS^{\cdot+}$) Scavenging Ability

The $ABTS^{\cdot+}$ scavenging ability of the aqueous extracts was determined according to the method described by Re *et al*. (1999). The $ABTS^{\cdot+}$ was generated by reacting an (7 mmol/L) ABTS aqueous solution with potassium peroxosulphate ($K_2S_2O_8$) (2.45 mmol/L, final concentration) in the dark for 16 hrs and adjusting the absorbance at 734 nm to 0.700 with ethanol. 0.2 mL of appropriate dilution of the extract was then added to 2.0 mL $ABTS^{\cdot+}$ solution and the absorbance was taken at 734 nm after 15 minutes. The trolox equivalent antioxidant capacity was subsequently calculated.

Nitric Oxide (NO) Radical Scavenging Ability

Nitric oxide (NO) scavenging assay was performed using Griess reagent method reported by Sufanta *et al*. (2006). Briefly, 1 mL each of various concentration of the extract (0.1 – 0.4 mg/mL) and 0.3 mL of sodium nitroprusside (5 mM) was added. The test-tubes were then incubated at 25°C for 150 minutes. 0.5 mL of Griess reagent (equal volume of 1% sulphanilamide on 5% autophosphoric acid and 0.01% naphthlethylmediamine in distilled water used after 12 hrs of preparation) was added. The absorbance was measured at 546 nm. Then, the percentage NO radical scavenging ability was subsequently calculated.

RESULTS

Figure 2 and Figure 3 respectively present the inhibitory effect of Avocado (*Persea americana*) pear leaves and fruit parts (leaves, peel, flesh and seed) on α-amylase and α-glucosidase. Extract concentration that will inhibit 50% enzyme activity (IC_{50}) was estimated and displayed in Table 1 below. In a dose dependent pattern (ranging from 0 – 0.164mg/mL), the result revealed that all the extracts inhibited α-amylase however, IC_{50} values (Table 1) revealed that the aqueous extract from the peel of avocado pear (IC_{50} = 0.28mg/mL) had the highest inhibitory effect on α-amylase activity while the seed (IC_{50} = 0.421mg/mL) had the least. The inhibitory strength of the aqueous extracts from Avocado pear (*Persea americana*) fruit parts and leaves was investigated on α-glucosidase activity and the result is presented in Figure 2. All the extracts inhibit α-glucosidase in a dose-dependent manner in the range of 0 – 0.4mg/mL with the peel (IC_{50} = 0.080mg/mL) having the greatest inhibitory potential while the leaf (IC_{50} = 0.636mg/mL) had the least.

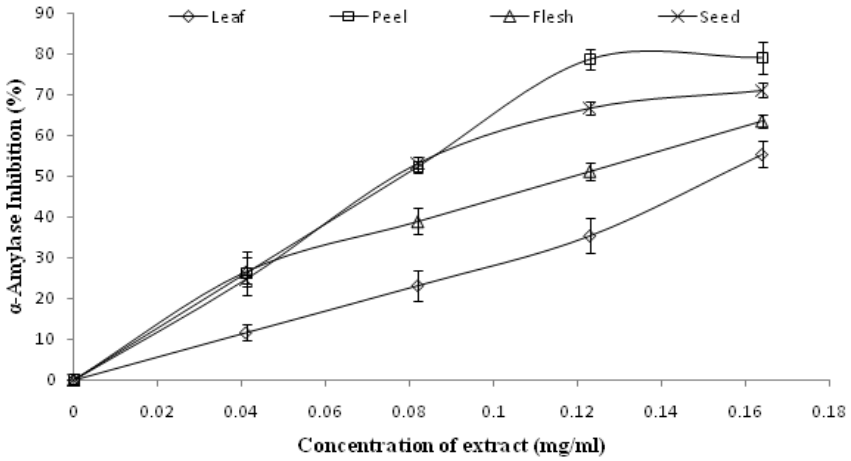

Figure 2: α-amylase Inhibitory Activity of *Persea americana* Leaves and Fruit Part Extracts

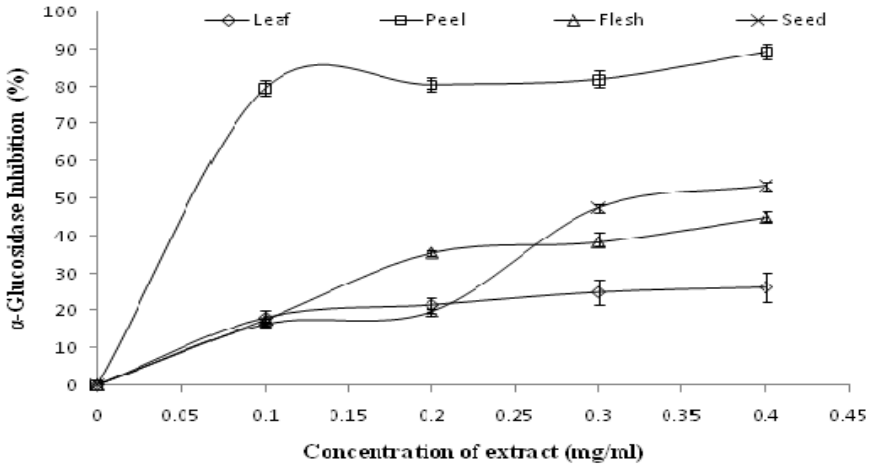

Figure 3: α-glucosidase Inhibitory Activity of *Persea americana* Leaves and Fruit Part Extracts

Table 1: IC_{50} (Extract Concentration Causing 50% Inhibitory Effect) Values of α-Amylase and α-glucosidase Inhibitory Activities by Aqueous Extract of Avocado Pear (*Persea americana*) Leaves and Fruit Parts

Samples	IC_{50} (mg/ml) α-amylase	IC_{50} (mg/ml) α-glucosidase
	Inhibitory Activity	Inhibitory Activity
Leaf	$0.280^{a} \pm 0.006$	$0.636^{a} \pm 0.021$
Peel	$0.172^{b} \pm 0.012$	$0.080^{b} \pm 0.006$
Flesh	$0.409^{c} \pm 0.021$	$0.559^{c} \pm 0.032$
Seed	$0.421^{c} \pm 0.010$	$0.364^{d} \pm 0.041$

Values represent Mean ± Standard deviation of triplicate readings. Values with the same superscript along the column are not significantly ($P < 0.05$) different.

Figure 3 shows a significant increase ($P < 0.05$) in the rat pancreas malondialdehyde (MDA) content when the rat's pancreas tissue homogenates was incubated in the presence of 7mM sodium nitroprusside (SNP). All the extracts inhibited malondialdehyde production content in the tissue in a dose-dependent manner (0 – 0.313mg/mL). Aqueous extract from the seed (0.059mg/mL) of avocado pear had the highest inhibitory effect on lipid peroxidation induced by sodium nitroprusside (a prooxidant) in the pancreas while the leaf (IC_{50} = 0.161mg/mL) had the least.

ABTS (2, 2^{1}–azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) free radical scavenging ability is presented as Trolox Equivalent Antioxidant Capacity (TEAC) in Figure 4. The results revealed that all the extracts scavenged ABTS free radical with the aqueous extract of the seed (12.997mmol. TEAC/100g) having the highest ABTS scavenging capacity while the flesh (8.372mmol. TEAC/100g) had the least.

NO (Nitric oxide) scavenging capacity of the aqueous extracts of leaves and fruit parts of Avocado (*Persea americana*) pear was investigated and the result presented in Figure 5. Judging with IC_{50} (extract concentration that will inhibit 50% oxidative potential of nitric oxide), it was revealed that the seed (IC_{50} = 0.059mg/mL) had the highest NO radical scavenging ability while the least NO radical inhibitory potential was exhibited by the leaf (IC_{50} = 0.289mg/mL).

In order to reveal the phytochemical constituents responsible for the antioxidant and inhibitory potentials of the aqueous extracts of Avocado (*Persea americana*) leaves and fruit parts on α-amylase, α-glucosidase and malondialdehyde (MDA) produced by SNP-induced lipid peroxidation, gas chromatography was carried out to characterize the aqueous extract. The result as revealed by Table 3 shows that syringic acid, eugenol, vnillic acid, kaemferol, catechin, epicatechin,

ferulic acid, apigenin, naringenin, lupeol, epigallocatechin and epigallocatechin-3-O-gallate are the major constituent of the leaves, peel flesh and seed extract of Avocado pear (*Persea americana*).

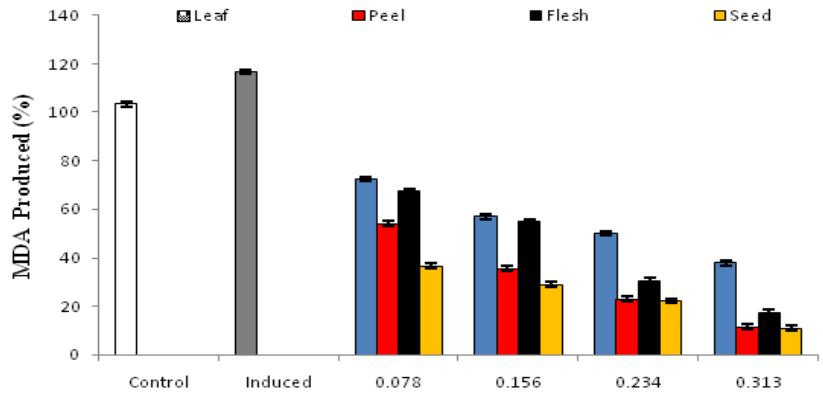

Figure 4: Inhibition of MDA Produced by SNP Induced Lipid Peroxidation by *Persea americana* Leaves and Fruit Part Aqueous Extracts

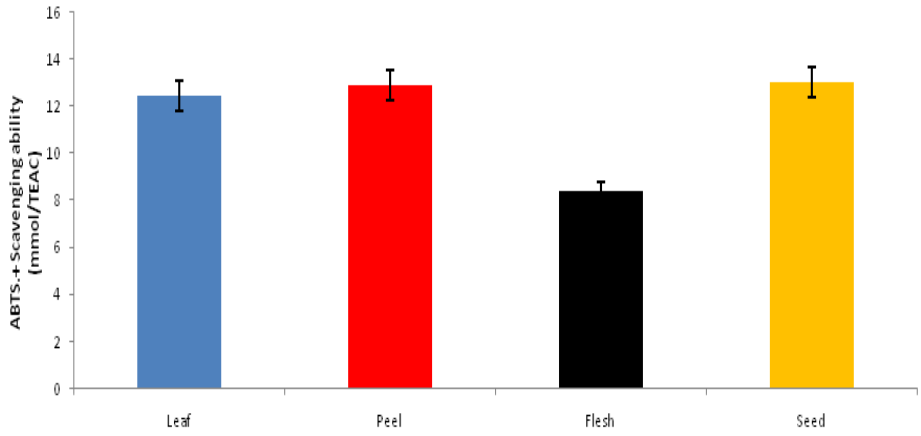

Figure 5: ABTS* Radical Scavenging Ability of Aqueous Extract of Leaves and Fruit Parts of *Persea americana*

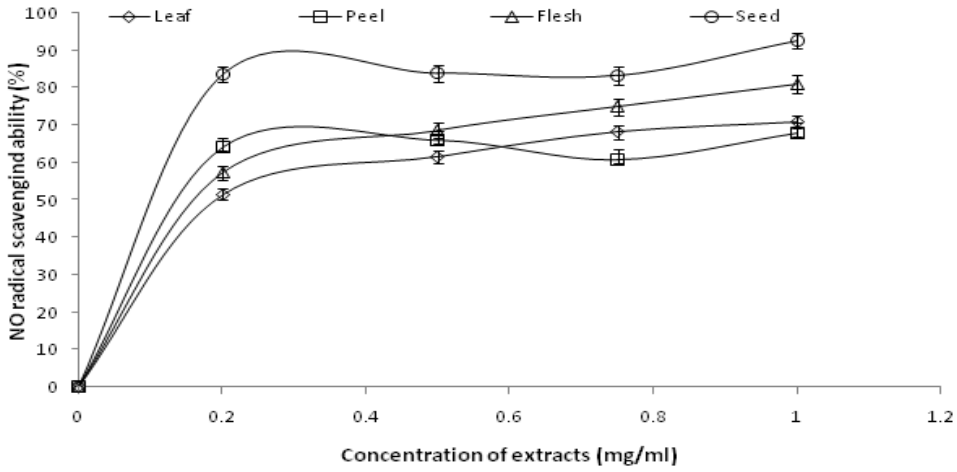

Figure 6: Nitric Oxide (NO) Radical Scavenging Ability of Aqueous Extracts of Leaves and Fruit Parts of Avocado (*Persea americana*)

Table 2: IC_{50} (Extract Concentration Causing 50% Inhibitory Effect) Values of No Radical Scavenging Ability and MDA Inhibitory Activity by Aqueous Extract of Avocado Pear (*Persea americana*) Leaves and Fruit Parts

Samples	IC_{50} (mg/ml) NO Scavenging Activity	IC_{50} (mg/ml) MDA Inhibitory Activity
Leaf	$0.289^{a} \pm 0.011$	$0.161^{a} \pm 0.008$
Peel	$0.257^{b} \pm 0.014$	$0.069^{b} \pm 0.005$
Flesh	$0.262^{b} \pm 0.007$	$0.122^{c} \pm 0.003$
Seed	$0.168^{c} \pm 0.006$	$0.059^{d} \pm 0.002$

DISCUSSIONS

One of the critical therapeutic strategies used to manage/control hyperglycemia is the inhibition of saccharide hydrolyzing enzymes (α-amylase and α-glucosidase) responsible for carbohydrate digestion (Shim *et al*., 2003). Pancreatic α-amylase catalyzes the incomplete breakdown of starch into disaccharides and oligosaccharides after which intestinal α-glucosidase catalyzes the complete breakdown of disaccharides to glucose which is been released into the blood stream. The slowing down of the breakdown of starch and absorption of glucose in the gastrointestinal tract through the inhibition of these enzymes can be exploited as a therapeutic measure to reduce postprandial hyperglycemia (Kwon *et al*., 2007). The search for natural α-glucosidase inhibitors with little or no side effects had been made attractive because of the severe gastrointestinal side effect attributed to synthetic α-glucosidase inhibitors such as acarbose and miglitol (Catherine *et al*., 2010).

First, the effect of *Persea americana* leaf and fruit parts (peel, and flesh seed) aqueous extract to inhibit α-amylase and α-glucosidase activities were investigated *in vitro* and the result is presented in Figure 1 and Figure 2 respectively. The results revealed that *P. americana* leaf and fruit parts inhibited α-amylase in a dose-dependent pattern. However, IC_{50} (extract concentration that inhibits 50% enzyme activity) values (Table 1) revealed that peel extract had a significant ($P < 0.05$) highest α-amylase and α-glucosidase inhibitory potentials. The seed and the leaf had the least inhibitory strength against α-amylase and α-glucosidase respectively. This research is in agreement with a recent work where red and white ginger inhibited α-amylase and α-glucosidase in vitro (Oboh *et al*., 2010) and the inhibition of saliva α-amylase activity by plant phytochemicals gotten from pepper (Aguilar-Santamaria *et al*., 2009; Oboh *et al*., 2011;). The free and bound phenol extracts of *Vernonia amygdalina del* significantly ($P < 0.05$) inhibited α-amylase and α-glucosidase activities *in vitro* in a dose-dependent pattern although the α-glucosidase inhibitory activity of the extracts were significantly ($P < 0.05$) higher than their α-amylase inhibitory activity (Saliu *et al*., 2011). This result also supports another where the seaweeds aqueous extracts in the order of *Gracilaria edulis* > *Sargassum polycystum* > *Ulva lactula* > *Gracilaria corticata* exhibited significant ($P < 0.05$) inhibitory activity against α-amylase and α-glucosidase enzymes (Palanisamy & Sellappa, 2012).

Conjugated dienes, malondialdehyde, 4-hydroxynonenal and others can be used as measures of injury mediated by free radical called lipid peroxidation (Denise *et al*., 2009). Oxidative stress increases the concentration of 2-thiobarbituric acid reactive substances (TBARS) which are naturally present in biological specimens (lipid hydroperoxides and aldehydes) (Armstrong & Browne, 1994). In this reaction, malondialdehyde (a compound that results from the decomposition of polyunsaturated fatty acid lipid peroxides) equivalent are normally been reported as TBARS assay values with criticism directed to the reactivity of thiobarbituric acid towards other compounds other than MDA (Yagi, 1998). Through knoevenagel-type condensation reaction, one molecule of MDA reacts with two molecules of 2-thiobarbituric acid to yield a chromophore with absorbance maximum at 532nm. The ability of the aqueous extract of *Persea americana* leaves and fruit parts to inhibit the production of malondiadehyde induced by sodium nitroprusside

(a prooxidant vasodilator that can produce nitric oxide spontaneously under appropriate reducing conditions) (James *et al*., 2010) was investigated and it was observed that all the extracts inhibited the production of malondialdehyde in a dose dependent manner. However, IC_{50} (extract concentration that will inhibit 50% MDA production) shows that the seed exhibited the highest significant ($P < 0.05$) inhibitory potential against malondialdehyde produced while the leaf showed the least inhibitory strength.

Comparing the result of TBARS assay with NO scavenging ability, it was observed that the seed had the highest significant ($P < 0.05$) nitric oxide (NO) radical scavenging ability which suggests the brain behind its ability to reduce the production of MDA. The contribution of NO radical to cardiovascular disease had been established due to their ability to react with reactive oxygen species (O_2^-) to give peroxinitrite ($ONOO^-$) that can induce lipid peroxidation and other complications that accumulatively lead to cell death (Parihar & Hemnani, 2004). This experiment supports another where oral supplementation of *Aphanamixis polystachya* bark at a daily dose of 50 and 100 mg body weight for 28 days exhibited significant reduction in hepatic MDA levels when compared with that of the disease control group. There was statistically significant ($P < 0.05$) inhibition of hepatic lipid peroxidation by all the doses used (Alluri *et al*., 2009). This work also supports another research in our laboratory where aqueous extracts of both white ginger (*Zingiber officinale* var. Roscoe) and red ginger (*Zingiber officinale* var. Rubra) inhibited sodium nitroprusside induced lipid peroxidation in the brain tissues (Oboh *et al*., 2012).

Table 3: Major Phenolics and Concentration (mg/100g) of Avocado (*Persea americana*) Pear Leaves and Fruit Parts

Phenolics	Leaves	Peel	Flesh	Seed
Syringic acid	31.65	$5.86e^{-4}$	$5.61e^{-4}$	27.38
Eugenol	21.52	$7.22e^{-4}$	$6.48e^{-4}$	14.53
Vnillic acid	13.33	$1.64e^{-3}$	14.54	10.46
Isoeugenol	12.92	$2.07e^{-4}$	$1.89e^{-4}$	10.73
Guaiacol	12.79	-	-	10.73
Phenol	8.47	-	-	10.13
Kaemferol	8.39	$4.48e^{-1}$	$8.68e^{-1}$	9.07
Catechin	-	4.39	-	-
P-hydroxybenzoic acid	$2.51e^{-4}$	1.37	1.24	$7.12e^{-4}$
Ferulic acid	$7.09e^{-5}$	8.92	11.07	$1.54e^{-4}$
Apigenin	$6.06e^{-5}$	2.13	2.84	$2.78e^{-4}$
Naringenin	$7.29e^{-4}$	3.20	1.86	$1.25e^{-3}$
Epigallocatechin	$2.31e^{-4}$	10.29	34.84	$3.06e^{-4}$
Epicatechin	$2.84e^{-4}$	37.12	46.82	$5.53e^{-4}$
Lupeol	$2.61e^{-6}$	10.89	19.73	$2.64e^{-6}$
Epi-gallocatechin-3-O-gallate	-	13.48	6.03	-

The importance of free radical scavengers in the management/treatment of disease conditions had been exploited in the production of herbal drugs (Patel *et al*., 2010). The mechanism of antioxidants on biological cells can either be by preventing the production of free radicals or by neutralizing/scavenging free radicals produced in the body (Oboh *et al*., 2007). The minimized spectra interference of $ABTS^{\cdot+}$ based model of free radical scavenging ability gives it advantage of been more versatile for the assessment of polar and non-polar samples at 760nm; a wavelength not normally encountered with natural products (Re *et al*., 1999). The total antioxidant capacity of the aqueous extracts was estimated as

Trolox Equivalent Antioxidant Capacity (TEAC) using a moderately stable nitrogen-centered radical species called $ABTS^{\cdot+}$ and presented in Figure 4. The result revealed that the seed had the highest $ABTS^{\cdot+}$ scavenging ability while the flesh had the least.

The Phenolic characterization of the leaves and fruit parts of avocado (*Persea americana*) as shown in Table 3 revealed that syringic acid was the key phenolics in leaves and seed while epigallocatechin was the major phenolics in peel and flesh samples.

CONCLUSIONS

The correlation between phenolic content of plant food and their antioxidant properties had been established (Sun *et al*., 2002). The major mechanisms of action of phenolics include reduction of free radical in order to stabilize them, activation of in vivo antioxidant enzymes, quenching of singlet oxygen, scavenging of free radicals, chelating metal catalysts, inhibition of oxidases and reduction of α-tocopherol radicals (Alia M *et al*., 2003; Amic D *et al*., 2003). One of the mechanisms used by these phenolics is strictly dependent on their ability to stabilize free radicals through the donation of proton (H^+) (Materska M & Perucka I, 2005). Therefore this work elucidates the mechanisms behind the ability of aqueous extracts of *Persea americana* leaves and fruit parts to inhibit α-amylase and α-glucosidase activities, ABTS free radical and NO radical scavenging ability and SNP-induced malondialdehyde produced by lipid peroxidation - The results shed light on the reason behind their effectiveness in disease management (specifically, diabetes type 2). The significant inhibitory prowess of *Persea americana* leaves and fruit parts aqueous extracts on α-amylase and α-glucosidase may be attributed to the high concentration of some phenolics revealed by the GC/MS which include syringic acid, eugenol, vnillic acid, isoeugenol, guaiacol, phenol, kaemferol, catechin, para-hydroxybenzoic acid, ferulic acid, apigenin, naringenin, epigallocatechin, epicatechin, lupeol and epi-gallocatechin-3-O-gallate. This research proves right the usage of *Persea americana* leaves and fruit parts in folk medicine for the management of diabetes type-2 and also established the mechanisms that might be responsible for this action. Therefore the result of this research suggests that avocado pear leaves can be used as nutraceuticals while the fruit can be functional food for the management of diabetes type-2 and its cardiovascular relatives.

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