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INHIBITORY EFFECT OF PHENOLIC EXTRACT FROM LEAF AND FRUIT OF AVOCADO PEAR (*PERSEA AMERICANA*) ON FE^{2+} INDUCED LIPID PEROXIDATION IN RATS' PANCREAS *IN VITRO*

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Abstract

Persea americana Mill (Luraceae) is one of the 150 varieties of avocado pear. The tree is widely cultivated in tropical and subtropical areas and has diverse application in ethno–medicine. This study sought to investigate the inhibitory effect of the phenolic extract of the leaves and fruit of *P. americana* on Fe^{2+} induced lipid peroxidation in rat's pancreas *in vitro*. The phenolics were extracted with 1M HCL and methanol solution (1:1), thereafter the total phenolic content and the antioxidant activities of the phenolic extracts were evaluated using various spectrophotometric methods. The results revealed that the Leaf had the highest total phenol content (43.82 mg GAE/g), flavonoid content (5.52 mg QUE/g) and DPPH radical scavenging ability while the Seed had the highest OH radical scavenging ability. Furthermore, all the extracts caused a significant decrease ($P<0.05$) in the MDA contents in the pancreas in a dose-dependent manner (0 – 175 µg/mL) with the seed (IC_{50} = 60.61 µg/mL) having the highest inhibitory effect on Fe^{2+} induced lipid peroxidation. In conclusion, the phenolic extract of the leaf and fruit parts of avocado pear (*P. americana*) were able to protect the pancreas from Fe^{2+} induced lipid peroxidation *in vitro*. Therefore, this protective effect of the phenolic extracts on Fe^{2+} induced lipid peroxidation could be attributed to their phenolic compound and, the mechanism through which they possibly do this, is by their Fe^{2+} chelating ability, radical scavenging abilities and reducing power.

Keywords: Lipid peroxidation; Malondialdehyde; Phenolic compounds; Antioxidant activities

INTRODUCTION

Oxidation of biological molecules has been postulated to induce a variety of pathological events such as diabetes, pancreatitis and ageing (Finkel and Holbrook, 2000). Evidence has shown that these damaging events are caused by free radicals (Halliwell and Gutteridge, 1993). Free radicals are unstable species because they have unpaired electron pairing with biological macromolecules. It is well established that free radicals are associated with process that leads to cell degeneration, especially in organs such as brain and pancreas (Shulman *et al*., 2004). Unchecked activities of ROS had been linked to health disorders such as diabetes mellitus, hypertension, cancer, neurodegenerative diseases, gastric ulcers, reperfusion, arthritis and inflammatory diseases (Halliwell, 1989; Vajragupta *et al*., 2000).

In the pancreas, Fe accumulates in acinar cells and in the islets of Langerhans, thereby resulting in the destruction of β-cells associated with diabetes mellitus (Shah and Fonseca, 2011). High levels of both Cu and Fe, with low levels of Zn and Mn play a crucial role in the progression of several degenerative diseases (Johnson, 2001). Although Fe is necessary physiologically as components of

many enzymes and proteins, free Fe in the cytosol and mitochondria could cause considerable oxidative damage by acting catalytically in the production of ROS which have the potential to damage cellular lipids, nucleic acids, proteins and carbohydrate resulting in wide-ranging impairment in cellular function and integrity (Britton *et al*., 2002). ROS can directly attack the polyunsaturated fatty acids of the cell membranes and induce lipid peroxidation. Malondialdehyde (MDA) is the end-product of lipid peroxidation, which is a process where reactive oxygen species (ROS) degrade polyunsaturated fatty acids. This compound is a reactive aldehyde and is one of the many reactive electrophile species that cause toxic stress in cells and form advanced glycation end-products. The production of this aldehyde is used as a biomarker to measure the level of oxidative stress in an organism (Murray *et al*., 2000).

However, the most likely and practical way to fight degenerative diseases is to improve body antioxidant status, which could be achieved by higher consumption of fruits and vegetables. Foods of plant origin usually contain natural antioxidants such as phenolic compounds that can scavenge free radicals (Sun *et al*., 2002; Alia *et al*., 2003; Oboh and Akindahunsi, 2004; Oboh, 2005).

Medicinal plant have continued to attract attention in the global search for effective methods of using plants' parts (e.g. seeds, stems, leaves, roots and bark etc) for the treatment of many diseases affecting humans (Sofowora, 1993). Many important drugs used in medicine to day are directly or indirectly derived from plants due to its bioactive constituents such as; alkaloids, steroids, tannins e.t.c (Cordeiro and Oniyangi, 1998). In recent years, secondary plant metabolites previously with unknown pharmacological activities have been extensively investigated as sources of medicinal agents (Krishnaraju *et al*., 2005). Phenolic compounds are an important group of secondary metabolites, which are synthesized by plants because of plant adaptation to biotic and a biotic stress condition such as infection, water stress, and cold stress (Oboh and Rocha 2007). In recent years, phenolic compounds have attracted the interest of researchers because of their antioxidants capacity; they can protect the human body from free radicals, whose formation is associated with the normal natural metabolism of aerobic cells. The antiradical activity of flavonoids and phenols is principally based on the structural relationship between different parts of their chemical structure (Rice-Evans *et al*., 1996).

Persea americana Mill (Luraceae) is one of the 150 varieties of avocado pear. The tree is widely cultivated in tropical and subtropical areas (Lu *et al*., 2005). The seed of *P. americana* (avocado seed) has diverse application in ethno–medicine, ranging from treatment for diarrhea, dysentery, toothache, intestinal parasites, skin treatment and beautification. The avocado seed oil has several health benefit e.g. for controlling human weight (especially used for obese for weight loss) (Lopez *et al*., 1996; Roger, 1999). *P. americana* leaves have been reported to have or posses anti inflammatory and analgesic activities (Adeyemi *et al*., 2002). The edible part (fruit) is very popular in vegetarian cuisine, making a substitute for meat in sandwiches and salads, because of its high fat content and high in valuable, health-promoting fats (Lu *et al*., 2005) . The fruit is not sweet but fatty, almost distinctly, yet subtly flavored, and of smooth, almost creamy texture. Avocado fruits in many countries such as Mexico, Brazil, South Africa and India are frequently used for milkshakes and occasionally added to ice-cream (Zeldes, 2010). While several works had been reported on the chemical characterization of phyto-constituents of *P. americana* fruit, there is still limited information on its potential use in the management/prevention of degenerative diseases associated with oxidative stress. Hence, the objective of this study is to investigate the antioxidative properties and inhibitory effect of phenolic extracts of the leaves and fruit parts of *P. americana* on Fe^{2+} induced lipid peroxidation in rats' pancreas – *in vitro*.

MATERIALS AND METHODS

Materials

Sample collection

The leaves and fruit of avocado pear (*Persea americana)* were obtained from a farm land at Ijoka, Akure and authentication of the plants were carried out by Mr. Segun in the Department of Crop, Soil and Pest Management, The Federal University of Technology, Akure, Nigeria. A voucher specimen was deposited at the Herbarium of Faculty of Science, Obafemi Awolowo University, Ile-Ife (OAU 2360). Ten adult male Wistar strain albino rats were purchased from the Animal Production and Health Department, Federal

University of Technology, Akure and acclimatized for 2 weeks, during which period they were maintained *ad libitum* on commercial diet and water. The handling of animals was carried out in accordance with the recommended international standard (National Research Council 1988). A UV-visible spectrophotometer (Model 6305; Jenway, Barlo world Scientific, Dunmow, United Kingdom) was used to measure absorbance.

Chemicals and reagents

Chemicals and reagents used such as thiobarbituric acid (TBA), 1,10-phenanthroline, deoxyribose, gallic acid, quecertin, ascorbic acid, Folin-Ciocalteau's reagent were procured from Sigma-Aldrich, Inc., (St Louis, MO), trichloroacetic acid (TCA), malonidialdehyde (MDA) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were sourced from Sigma-Aldrich, Chemie GmbH (Steinheim, Germany), hydrogen peroxide, methanol, acetic acid and HCl were sourced from BDH Chemicals Ltd., (Poole, England), sodium carbonate, $AlCl_3$, potassium acetate, Tris-HCl buffer, sodium dodecyl sulphate (SDS), $FeSO_4$, potassium ferricyanide and ferric chloride were of analytical grade while the water was glass distilled.

Extraction of Phenolics

The leaves and fruits were rinsed with distilled water after which the peel, flesh and seed were chopped into pieces and air-dried before milling into a fine powdery form. Then, the total phenolics of the samples were extracted with 1M HCL and methanol solution (1:1 v/v) and filtered (filter paper Whatman number 2) under vacuum. The filtrate was then evaporated using a rotary evaporator under vacuum at 45°C. Then, the phenolics extracts were reconstituted in distilled water (1:100 w/v) and stored in the refrigerator for subsequent analysis.

Determination of phenol content

The total phenol content was determined on the unripe pawpaw fruit parts using the method reported by Singleton *et al*. (1999). Appropriate dilutions of the extracts were mixed with 2.5 mL of 10% Folin–Ciocalteau's reagent (v/v) and neutralized by 2.0 mL of 7.5% sodium carbonate. The reaction mixture was incubated for 40 min at 45°C and the absorbance was measured at 765 nm. The total phenol content was subsequently calculated using gallic acid as standard.

Determination of flavonoid content

The flavonoid contents of *P. americana* leaves and fruit parts were determined using a slightly modified method reported by Meda *et al*. (2005). Briefly, 0.5 mL of appropriately diluted sample was mixed with 0.5 mL methanol, 50 µL of 10% $AlCl_3$, 50 µL of 1 M potassium acetate and 1.4 mL water, and allowed to incubate at room temperature for 30 min. Thereafter, the absorbance of the reaction mixture was subsequently measured at 415 nm. The total flavonoid was calculated using quercetin as standard.

Preparation of pancreas homogenates

The rats were decapitated under mild diethyl ether anesthesia and the pancreas tissue was rapidly dissected and placed on ice and weighed. This tissue was subsequently homogenized in cold saline (1:10 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 (S1) containing mainly water, proteins and lipids (cholesterol, galactolipid, individual phospholipids, gangliosides) was kept for lipid peroxidation assay (Belle *et al*., 2004).

Lipid peroxidation and thiobarbibutric acid reactions

The lipid peroxidation assay was carried out using the method of Ohkawa *et al*. (1979). Briefly 100 µL S1 fraction was mixed with a reaction mixture containing 30 µL of 0.1 M Tris-HCl buffer (pH 7.4), extract of unripe pawpaw fruit parts (0-100 µL) and 30 µL of 250 µM freshly prepared $FeSO_4$. The volume was made up to 300 µL with water before incubation at 37°C for 1 hr. The color reaction was developed by adding 300 µL, 8.1% SDS to the reaction mixture containing S1; this was subsequently followed by the addition of 500 µL of acetic acid/HCl (pH 3.4) and 500 µL, 0.8% TBA. This mixture was incubated at 100°C for 1 hr. The absorbance of thiobarbituric acid reactive species produced was measured at 532 nm. Malondialdehyde (MDA) produced was expressed as % Control, The IC_{50} (extract concentration required to inhibit 50% of MDA produced) values were calculated using non-linear regression analysis.

Fe^{2+} chelation assay

The Fe^{2+} chelating ability of the phenolic extracts were determined using a modified method of Minotti and Aust (1987) with a slight modification

by Puntel *et al*. (2005). Freshly prepared 500 µM $FeSO_4$ (150 µL) was added to a reaction mixture containing 168 µL of 0.1 M Tris-HCl (pH 7.4), 218 µL saline and the extracts (0 - 25 µL). The reaction mixture was incubated for 5 min, before the addition of 13 µL of 0.25% 1,10-phenanthroline (w/v). The absorbance was subsequently measured at 510 nm. The Fe^{2+} chelating ability was subsequently calculated with respect to the reference.

$$[(Abs_{ref} - Abs_{sample})/ Abs_{ref}] \times 100$$

Where,

Abs_{ref} = Absorbance of the reference (reacting mixture without the test sample) and,

Abs_{sample} = Absorbance of reacting mixture with the test sample

Degradation of deoxyribose (Fenton's reaction)

The ability of the phenolic extracts to prevent Fe^{2+}/H_2O_2 induced decomposition of deoxyribose was carried out using the method of Halliwell and Gutteridge (1981). Briefly, freshly prepared aqueous extract (0 - 100 µL) was added to a reaction mixture containing 120 µL 20 mM deoxyribose, 400 µL 0.1 M phosphate buffer, 40 µL 20 mM hydrogen peroxide and 40 µL 500 µM $FeSO_4$, and the volume for made to 800 µL with distilled water. The reaction mixture was incubated at 37°C for 30 min, and the reaction was stop by the addition of 0.5 mL of 2.8% TCA, this was followed by the addition of 0.4 mL of 0.6% TBA solution. The tubes were subsequently incubated in boiling water for 20 min. The absorbance was measured at 532 nm.

$$[(Abs_{ref} - Abs_{sample})/ Abs_{ref}] \times 100$$

Where,

Abs_{ref} = Absorbance of the reference (reacting mixture without the test sample) and,

Abs_{sample} = Absorbance of reacting mixture with the test sample

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

The free radical scavenging ability of the phenolic extracts against 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was evaluated as described by Gyamfi *et al*. (1999). Briefly, appropriate dilution of the extracts (1 mL) was mixed with 1 mL, 0.4 mM methanolic solution containing DPPH radicals, the mixture was left in the dark for 30 min and the absorbance was taken at 516 nm. The DPPH free radical scavenging ability was subsequently calculated.

$$[(Abs_{ref} - Abs_{sample})/ Abs_{ref}] \times 100$$

Where,

Abs_{ref} = Absorbance of the reference (reacting mixture without the test sample) and,

Abs_{sample} = Absorbance of reacting mixture with the test sample

Reducing property

The reducing properties of all the extracts were determined by assessing the ability of the extract to reduce a $FeCl_3$ solution as described by Pulido *et al*. (2000). A 2.5 mL aliquot was mixed with 2.5 mL, 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL, 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min, and then 2.5 mL, 10% TCA was added and centrifuged at 650 g for 10 min. A 5 mL of the supernatant was mixed with an equal volume of water and 1 mL, 0.1% ferric chloride. The same treatment was performed to a standard ascorbic acid solution and the absorbance taken at 700 nm. The reducing power was then calculated and expressed as ascorbic acid equivalent.

Data analysis

The result of 3 replicate experiments were pooled and expressed as mean ± standard deviation (SD) (Zar, 1984). A one-way analysis of variance (ANOVA) and the least significance difference (LSD) were carried out. Significance was accepted at $P \leq 0.05$.

RESULTS AND DISCUSSION

Medicinal plant have continued to attract attention in the global search for effective methods of using plants' parts (e.g. seeds, stems, leaves, roots and bark etc) for the treatment of many diseases affecting humans (Sofowora, 1993). Many important drugs used in medicine today are directly or indirectly derived from plants due to its bioactive constituents. Many plants are rich sources of phytochemicals, and intakes of these plant chemicals have protective potential against degenerative diseases (Chu *et al*., 2002). The total phenol content of the leaves and fruit parts of avocado pear (*Persea americana*) is presented in Table 1. The result revealed that the leaf (43.82 mg GAE/g) had the highest total phenol content followed by the peel (30.01 mg GAE/g) while the Flesh (16.81 mg GAE/g) had the least. Phenolic compounds can protect the human body from free radicals, whose formation is associated with the normal metabolism of aerobic cells. They are strong antioxidants capable of removing free

radicals, chelate metal catalysts, activate and inhibit oxidases (Amic *et al*., 2003). antioxidant enzymes, reduce α-tocopherol radicals

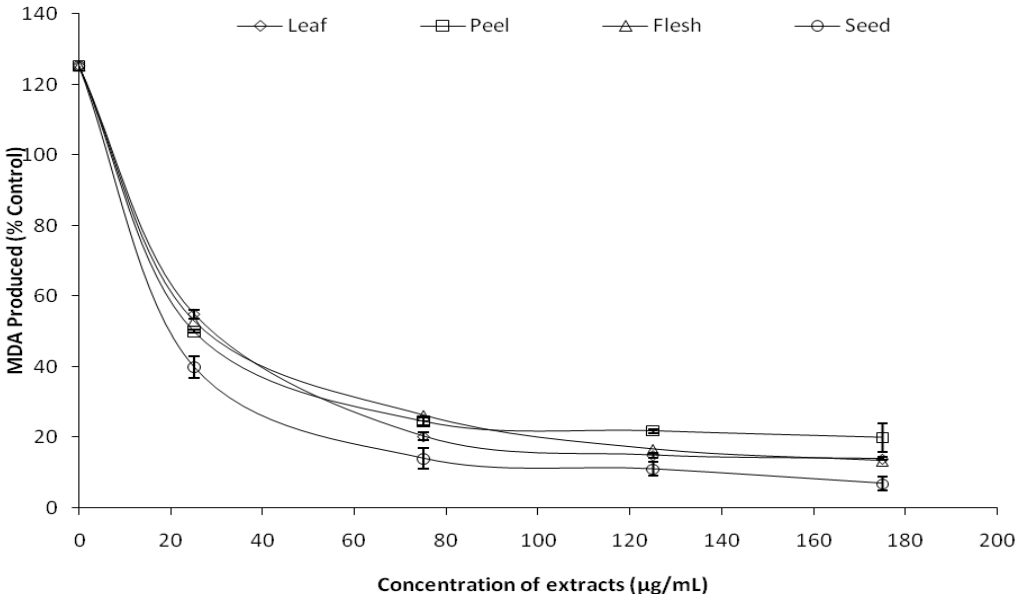

Figure 1: Inhibition of Fe^{2+}- Induced Lipid Peroxidation in rat's pancreas by phenolic extract of leaves and fruit parts of avocado pear (*Persea americana*)

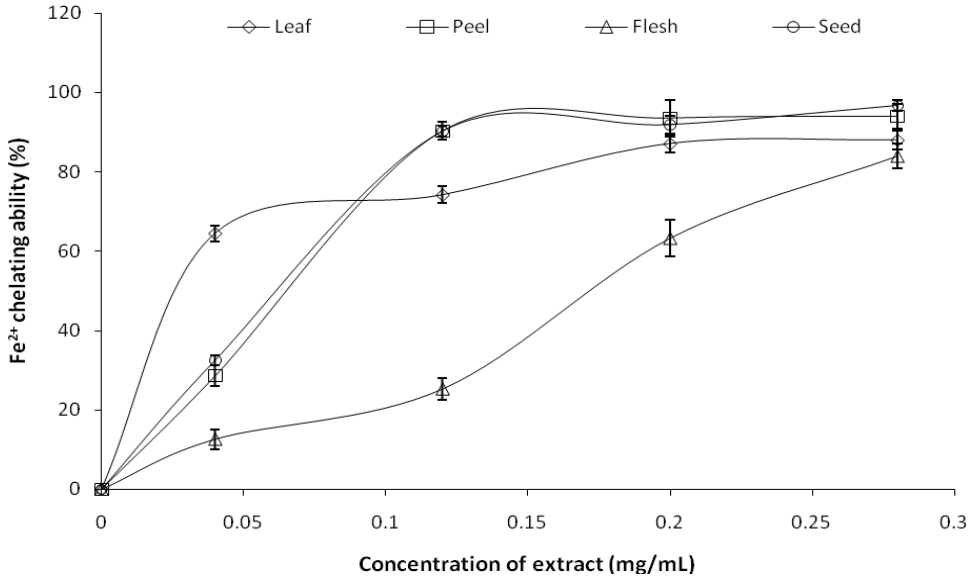

Figure 2: Fe^{2+} Chelating ability of phenolic extract of leaves and fruit parts of avocado pear (*Persea americana*)

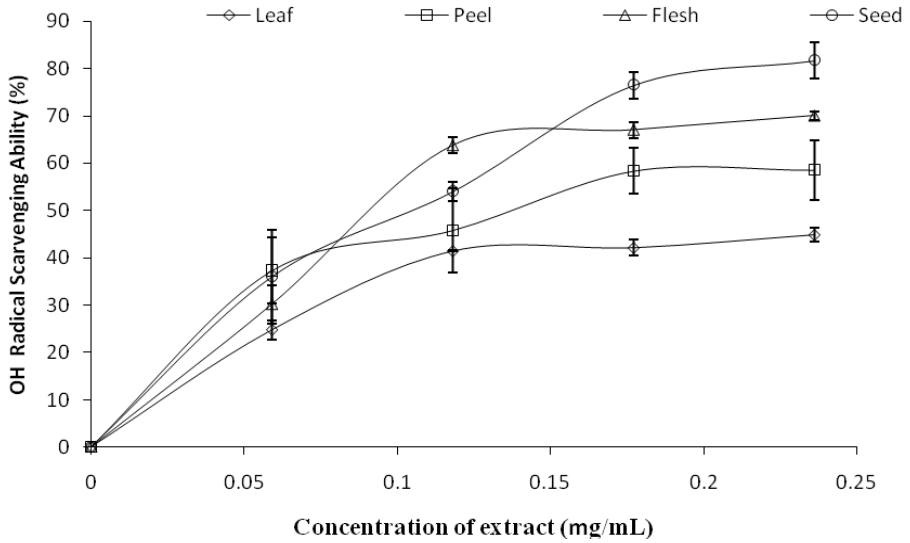

Figure 3: OH radical scavenging ability of phenolic extract of leaves and fruit parts of avocado pear (*Persea americana*)

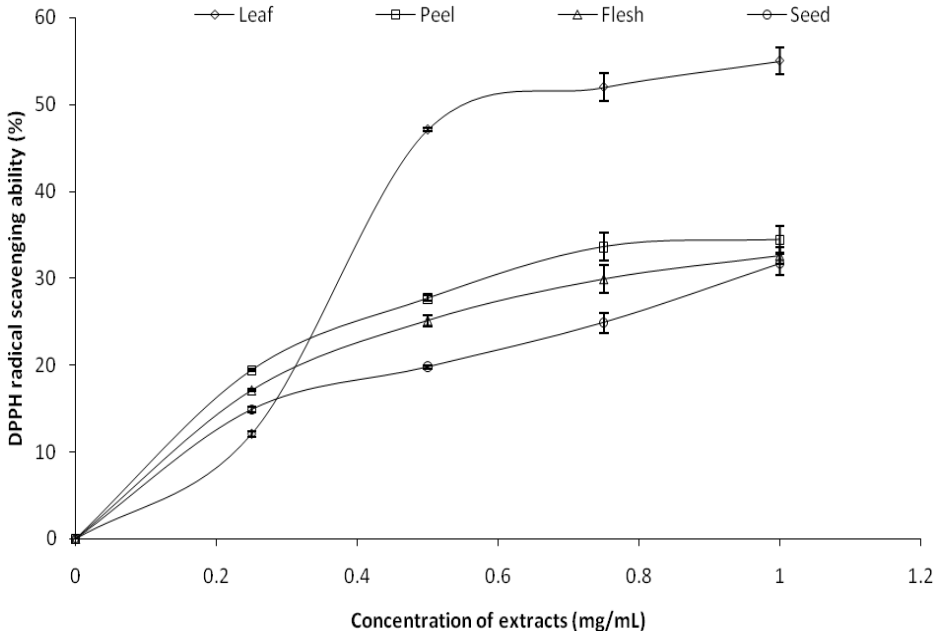

Figure 4: DPPH Free radical scavenging ability of phenolic extract of leaves and fruit parts of avocado pear (*Persea americana*)

Table 1: Total Phenol content, Total Flavonoid content and Ferric Reducing Antioxidant Property (FRAP) of leaves and fruit parts of avocado pear (*Persea americana*)

Sample	Total phenol (mg GAE/g)	Total flavonoid (mg QUE/g)	FRAP (mg. AAE/g)
Leaf	$43.82^{a} \pm 0.035$	$5.52^{a} \pm 0.040$	$29.52^{b} \pm 0.055$
Peel	$30.01^{b} \pm 0.065$	$3.39^{b} \pm 0.035$	$34.64^{a} \pm 0.140$
Flesh	$16.81^{c} \pm 0.030$	$3.46^{d} \pm 0.040$	$24.91^{c} \pm 0.030$
Seed	$29.37^{d} \pm 0.035$	$2.32^{c} \pm 0.040$	$27.70^{b} \pm 0.145$

Values represent Mean ± Standard deviation of triplicate readings
Values with the same superscript along the column are not significantly (P<0.05) different
KEY:
AAE - Ascorbic acid equivalent
GAE - Gallic acid equivalent
QUE - Quercetin equivalent

Table 2: IC_{50} (extract concentration causing 50% inhibitory effect) values of phenolic extract of leaves and fruit parts of avocado pear (*Persea americana*) on Fe^{2+} Induced Lipid Peroxidation in rat's pancreas

Sample	IC_{50} of Fe^{2+} induced lipid peroxidation in rat pancreas (μg/mL)
Leaf	$72.47^{a} \pm 2.25$
Peel	$76.67^{b} \pm 5.03$
Flesh	$74.28^{a} \pm 6.05$
Seed	$60.61^{c} \pm 2.84$

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

Furthermore, the total flavonoid content of the leaf and fruit parts of avocado pear (*P. americana*) as presented in Table 1 revealed that leaf (5.52 mg QUE/g) had the highest flavonoid content while the seed (2.32 mg QUE/g) had the least. The presence of derivatives of flavonoids has been found in many herbs and fruits; moreover, numerous studies have conclusively shown that the majority of the antioxidant activity maybe from compounds such as flavonoids, isoflavones, flavones, anthocyanins, catechin and isocatechin rather than from vitamins C, E and β-carotene (Marin *et al*., 2004; Oboh *et al*., 2007). Flavonoids have antioxidant activity and could therefore lower cellular oxidative stress (Oboh *et al*., 2007). Polyphenols are considered to be strong antioxidants due to the redox properties of their hydroxyl groups (Materska and Perucka 2005).

The finding that Fe^{2+} caused a significant increase in the MDA content of the pancreas agreed with earlier report where Fe^{2+} was shown to be a potent initiator of lipid peroxidation (Oboh *et al*., 2007). The increased lipid peroxidation in the presence of Fe^{2+} could be attributed to the fact that Fe^{2+} can catalyze one-electron transfer reactions that generate reactive oxygen species, such as the reactive $OH^{\bullet}$, which is formed from H_2O_2 through the Fenton reaction. This $OH^{\bullet}$ later attacks DNA, protein, membrane lipids, and several other biomolecules of physiological importance. Iron also

decomposes lipid peroxides, thus generating peroxyl and alkoxyl radicals, which favors the propagation of lipid oxidation (Zago *et al*., 2000). In the pancreas, Fe accumulates in acinar cells and in the islets of Langerhans, thereby resulting in the destruction of β-cells associated with diabetes mellitus (Shah and Fonseca, 2011). Therefore, possible depletion of iron could decrease oxidative stress throughout the whole body (Minamiyama *et al*., 2010). However, the phenolic extract of the leaf and fruit parts of avocado pear (*P. americana*) caused a dose-dependent significant decrease ($P<0.05$) in the MDA content of the Fe^{2+}-stressed pancreas homogenates (Figure 1). Nevertheless, phenolic extract of the seed (IC_{50} = 60.61 µg/mL) had the highest inhibitory effect on Fe^{2+} induced lipid peroxidation in rat's pancreas followed by leaf (IC_{50} = 72.47 µg/mL) while the peel (IC_{50} = 76.67 µg/mL) had the least when taking into account the IC_{50} values in Table 2. The mode of inhibition of Fe^{2+} induced lipid peroxidation cannot be categorically stated, however there is the possibility that the phenolics could have formed complexes with the Fe^{2+} thereby preventing them from catalyzing the initiation of lipid peroxidation and/or the possibility that the phytochemical could have scavenge the free radical produced by the Fe^{2+}-catalyzed reaction (Oboh *et al*., 2007).

In an attempt to explain the possible mechanism by which the leaf and fruit of avocado pear (*P. americana*) protect the pancreas from Fe^{2+} induced lipid peroxidation. The Fe^{2+} chelating ability of the aqueous extracts of the leaf and fruit of avocado pear (*P. americana*) was assessed (Figure 2). The result revealed that all the extracts were able to chelate Fe^{2+} in a dose dependent manner (0 – 0.28 mg/mL). However, phenolic extract of the leaf had the highest Fe^{2+} chelating ability at lower concentration while at higher concentration the seed had the highest Fe^{2+} chelating ability. The ability of substances to chelate and deactivate transition metals, prevent such metals from participating in the initiation of lipid peroxidation and oxidative stress through metal catalysed reaction is considered an antioxidant mechanism of action (Oboh *et al*., 2007). The functional groups present in the extracts such as -OH, -SH, -COOH, PO_3H_2, C=O, $-NR_2$, -S- and -O- may be responsible for the Fe^{2+} chelating ability of the extract (Lindsay, 1996; Yuan *et al*., 2005; Gülçin, 2006). Earlier reports revealed that compounds with structures containing two or more of the following functional groups: -OH, -SH, -COOH, PO_3H_2, C=O, $-NR_2$, -S- and -O- in a favourable structure-function configuration can show metal chelation activity (Lindsay, 1996; Yuan *et al*., 2005; Gülçin, 2006). This high Fe^{2+}chelating ability of the phenolic extract is of immense importance in the protective ability of polyphenol against oxidative stress, because it is usually too late to attempt to use OH radical scavengers for therapeutic purposes. The reason for this is that extraordinarily high reactivity of hydroxyl radicals towards most biomolecules would require unreasonably high concentrations of intercepting scavengers to outcompete the biomolecules of interest (Bayir *et al*., 2006), thereby making Fe^{2+} chelators a better therapeutic alternative. The hydroxyl radical ($OH^{\bullet}$) scavenging ability of the phenolic extracts of the leaf and fruit of avocado pear (*P. americana*) is presented in Figure 3. The results revealed that all the extracts were able to scavenge $OH^{\bullet}$ produced from the decomposition of deoxyribose in Fenton reaction in a dose-dependent manner (0 0.236 mg/mL). However, the seed had the highest OH radical scavenging ability while the lea had the least.

Antioxidants carry out their protective role on cells either by preventing the production of free radicals or by neutralizing/scavenging free radicals produced in the body (Alia *et al*., 2003; Amic *et al*., 2003; Oboh *et al*., 2007). The result of the DPPH radical scavenging ability of the extract as presented in Figure 4 revealed that all the extracts scavenged DPPH radicals in a dose-dependent pattern (0 – 1.0 mg/mL). However, the leaf (48.60 - 81.63%) had the highest DPPH radical scavenging ability while the flesh (18.72 - 37.79%) had the least. The DPPH radical scavenging ability of the phenolic extracts could be attributed to the hydrogen donating ability of the hydroxyl groups of the phenolics. The radical scavenging ability of phenolics is attributed to the hydroxyl groups and the availability of hydrogen for donation (Chu *et al*., 2002). Reducing power is a potent antioxidation defence mechanism. The two mechanisms that are available to affect this reducing power are by electron transfer and hydrogen atom transfer (Dastmalchi *et al*., 2007). This is because the ferric-to-ferrous ion reduction occurs rapidly with all reductants with half reaction reduction potentials above that of Fe^{3+}/Fe^{2+}, the values in the Ferric

reducing antioxidant property (FRAP) assay will express the corresponding concentration of electron-donating antioxidants (Halvorsen *et al*., 2002). The results revealed that the peel (34.64 mg AAE/g) had the highest reducing power while the flesh (24.91 mg AAE/g) had the least. Phenolic compounds have been reported to possess a higher reducing power than classical antioxidants such as BHA, BHT, tocopherol and trolox (Gülçin, 2006). The high reducing power of the phenolic extracts will be of immense advantage in neutralizing free radicals generated in hyperglycaemic condition associated with diabetes thus slowing down the development of diabetic complications arising from oxidative stress.

CONCLUSION

In conclusion, the phenolic extract of the leaf and fruit parts of avocado pear (*P. americana*) were able to protect the pancreas from Fe^{2+} induced lipid peroxidation *in vitro*. Therefore, the protection of pancreas tissue from Fe^{2+} induced lipid peroxidation by phenolic extract of the leaf and fruit parts of avocado pear (*P. americana*) could be attributed to their phenolic compound and, the mechanism through which they possibly do this, is by their Fe^{2+} chelating ability, radical scavenging abilities and reducing power.

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