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Inhibitory Effect of Aqueuos Extracts of Avocado Pear (*Persea americana*) Leaf and Seed on Angiotensin 1- Converting Enzyme: A Possible Means in Treating/Managing Hypertension

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Authors' contributions

This work was carried out in collaboration between all authors. Author GO designed the study and wrote the protocol. Author VOO wrote the first draft of the manuscript, managed part of the literature searches, analyses of the study, performed the spectroscopy analysis and managed the experimental process and author OAM concluded the manuscript writing and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study sought to investigate the inhibitory effect of aqueous extract of Avocado pear leaf and seed on angiotensin 1 converting enzyme activity.
Place and Duration of Study: The work was carried out at The Neutraceutical and Pytomedicine Laboratory Unit of The Federal University of Technology Akure, Nigeria between October 2011 and February 2012.
Methodology: The aqueous extract of the samples were prepared (1:10 w/v) and were used for subsequent analysis. The total phenolic content and antioxidant properties of the leaf and seed of Avocado pear were evaluated. Thereafter, the inhibitory effect of the extracts on angiotensin 1 converting enzyme was determined *in vitro.*
Results: The results revealed that the leaf had significantly (P<0.05) higher total phenol (92.9 mg/g) and flavonoid content (51.0 mg/g) than the seed [total phenol (57.1 mg/g) and total

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flavonoid (19.48 mg/g) content]. Furthermore, the antioxidant properties of the leaf [as typified by ABTS] was also significantly (P<0.05) higher than that of the seed. Conversely, the seed had higher inhibitory effect on angiotensin 1 converting enzyme than the leaf extract.
Conclusion: The inhibitory effect of the avocado pear leaf and seed could be due to some phytochemicals present in the extracts. These antioxidant and enzyme inhibition of the leaf and seed of avocado pear could be part of the mechanism for their use in folklore medicine in the management/treatment of hypertension. However, the seed had a better antihypertensive property while the leaf had a better antioxidant property.

Keywords: Avocado pear; Angiotensin-1 converting enzyme; hypertension; antioxidant.

ABBREVIATIONS

ACE - Angiotensin -1- converting enzyme;
EC_{50} - Extract concentration causing 50% enzyme inhibition

1. INTRODUCTION

Hypertension, referred to as high blood pressure, is a medical condition in which the blood pressure is chronically elevated [1]. Persistent hypertension is one of the risk factors for strokes, heart attacks, heart failure and is a leading cause of chronic renal failure [2,3]. Hypertension has become a worldwide problem of epidemic proportions, affecting 15–20% of all adults with ailments such as arteriosclerosis, stroke, myocardial infarction and end-stage renal disease [1]. Development of atherosclerotic diseases have been linked to oxidative stress which is the steady level of oxidative damage in a cell, tissue, or organ, caused by reactive oxygen species (ROS). This damage can affect a specific molecule or the entire organism. ROS, such as free radicals and peroxides, represent a class of molecules produced during the metabolism of oxygen and exist inherently in all aerobic organisms [4]. The level of oxidative stress is a balance between the rate at which oxidative damage is induced (input) and the rate at which it is removed (output) [5]. The brain and nervous system are particularly vulnerable to oxidative stress because of limited antioxidant capacity [6]. The brain makes up about 2% of a person's mass but consumes 20% of an individual's metabolic oxygen. The vast majority of this energy is used by the neurons [6]. Some brain cells, like neurons, cannot produce glutathione, but instead rely on surrounding astrocyte cells to provide usable glutathione precursors. Because the brain has limited access to the bulk of antioxidants produced by the body, neurons are the first cells to be affected by a shortage of antioxidants and are most susceptible to oxidative stress [7]. The involvement of free radical reactions in the pathogenesis of heart diseases has been investigated for many years. It is now generally accepted that reactive free radicals can exert cellular damage through a variety of mechanisms, *e.g*., lipid peroxidation, covalent binding, depletion of glutathione and protein thiols, derangement of intracellular free calcium homeostasis, DNA fragmentation, etc., with different relevance in the various conditions [8]. Free radical damage to DNA and its effect on LDL cholesterol is very likely responsible for heart disease. An essential involvement of lipid peroxidation in the events leading to hypertension has been proved *in vitro* and *in vivo* in the presence of various pro-oxidants. Studies on atherosclerosis reveal the probability that the disease may be due to free radical reactions involving diet-derived lipids in the arterial wall and serum to yield peroxides and other substances. These compounds induce endothelial cell injury and produce changes in the arterial walls [9]. The modification of lipid concentration has been found to be a useful approach to decrease cardiovascular mortality through prevention of development of atherosclerotic diseases [10-12]. The inhibition of Angiotensin-1 converting enzyme (ACE) has been suggested to be a useful approach to the management and prevention of hypertension; and dietary phenolics have promising potential [13]. Angiotensin is a potent vasoconstrictor, but also increases activity of the sympathetic nervous system by both central and peripheral mechanisms [14]. In humans, the rennin-angiotensin system (RAS) plays a pivotal role in blood pressure regulation, and in the pathophysiology of cardiovascular diseases such as congestive heart failure and hypertension [15]. Renin produces angiotensin I from angiotensinogen, after which it is cleaved by angiotensin I converting enzyme (ACE) to release angiotensin II, a potent vasoconstrictor

[16]. ACE also inactivates bradykinin, which has depressor action [17]. Inhibition of the angiotensin-1 converting enzyme (ACE) is established as a modern therapeutic principle in the treatment of hypertension [16]. It is also know that Inhibition of ACE activity has been associated with both phenolic and flavonol contents in foods [18].

The Avocado pear *(Persea americana),* is a tree native to Mexico and Central America, classified in the flowering plant family Lauraceae and widely cultivated in subtropical regions for its large, edible fruit. It is characterized by an oval or pear-shape, with a rough or leathery skin, and a large seed; it is rich in vitamins, high in monounsaturated fat and potassium, and containing a unique fatty alcohol, avocadene, avocado fruits provide curative effects for a number of human ailments, from diarrhea to high blood pressure. A whole medium avocado pear contains approximately 55 percent of the United States FDA's recommended daily amount of fat, though they are high in monounsaturated fat. Avocados also have 60 percent more potassium than bananas. They are rich in B vitamins, as well as vitamin E and vitamin K [19]. A fatty triol (fatty alcohol) with one double bond, avocadene (16-heptadecene-1,2,4-triol), is found in avocado and has been tested for anti-bacterial and anti-inflammatory properties. These properties are likely related with the curative effects of avocado pear described for high blood pressure [20]. Avocado Pear can also be beneficial in controlling blood cholesterol levels, and increasing urine acidity. In ancient Greece, Avocado pears were used to treat nausea. Most of the fiber is insoluble, making Avocado pear a good laxative [21]. Because of the medicinal properties of the *P. americana* leaf and seed, they have received attention from phytochemists and food scientists; however, there is limited information with regard to its ability to prevent/treat hypertension. This study therefore sought to determine the inhibitory effect of aqueous extracts of *P. americana* leaf and seed on angiotensin-1 converting enzyme *in vitro* and to determine which of the part is more potent in the management and prevention of hypertension.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Sample collection

The pear (*Persea americana*) leaves and seed were collected from a garden in Akure, Ondo State. Authentication of the samples was carried out at the Department of Biology, Adekunle Ajasin University, Akungba Akoko, Nigeria.

2.1.2 Aqueous extract preparation

The samples were washed under running water while the seeds were separated from the flesh, chopped into small pieces by table knife, air dried and milled. The aqueous extract of the samples were subsequently prepared by soaking the powdered samples in water (1:10 w/v) for about 24 h at 37℃, the mixture was filtered and later centrifuged to obtain a clear supernatant which was then stored in the refrigerator for subsequent analysis [4].

2.1.3 Determination of total phenol content

The total phenol content was determined according to the method of [22]. Briefly, appropriate dilutions of the extracts were oxidized with 2.5ml 10% Folin-Ciocalteau's reagent (v/v) and neutralized by 2.0ml of 7.5% sodium carbonate. The reaction mixture was incubated for 40 minutes at 45^{o}C and the absorbance was measured at 765nm in the spectrophotometer. The total phenol content was subsequently calculated as gallic acid equivalent.

2.1.4 Determination of total flavonoid content

The total flavonoid content was determined using a slightly modified method reported by Meda [23], briefly 0.5ml of appropriately diluted sample was mixed with 0.5ml methanol, 50µl 10% $AlCl_3$, 50µl 1M Potassium acetate and 1.4ml water, and allowed to incubate at room temperature for 30 minutes. The absorbance of the reaction mixture was subsequently measured at 415 nm; the total flavonoid content was subsequently calculated. The non-flavonoid polyphenols were taken as the difference between the total phenol and total flavonoid content.

2.1.5 Phytochemical screening

The methods described by Aiyegoro [24] were used for phytochemical screening of the aqueous extracts for the presence of bioactive compound.

2.1.6 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) Radical Scavenging Ability

The ABTS* scavenging ability of the extracts were determined according to the method

described by Re [25]. The ABTS* was generated by reacting an (7 mmol/l) ABTS aqueous solution with $K_2S_2O_8$ (2.45 mmol/l, final concentration) in the dark for 16 h and adjusting the Abs 734 nm to 0.700 with ethanol. 0.2 ml of appropriate dilution of the extract was added to 2.0 ml ABTS* solution and the absorbance were measured at 734 nm after 15 minutes. The trolox equivalent antioxidant capacity was subsequently calculated.

2.1.7 Angiotensin -1 converting enzyme (ACE) inhibition assay

Appropriate dilution of the aqueous extracts (50 µl) and ACE solution (50 µl, 4 mU) was incubated at 37℃ for 15 min. The enzymatic reaction was initiated by adding 150 µl of 8.33 mM of the substrate Bz–Gly–His–Leu in 125 mM Tris– HCl buffer (pH 8.3) to the mixture. After incubation for 30 minutes at 37℃, the reaction was arrested by adding 250 µl of 1 M 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 re-dissolved in distilled water and its absorbance was measured at 228 nm. The ACE inhibitory activity was expressed as percentage inhibition. The EC_{50} (the extract concentration inhibiting 50% of the ACE activity) of the aqueous extracts were calculated [26].

2.1.8 Data analysis

The results of the aqueous extract of the pear leaf and seed were pooled and expressed as mean ± standard error (S.E.). Student t-test, one-way analysis of variance (ANOVA) and the least significance difference (LSD) were carried out [27]. Significance was accepted at P =.05. EC_{50} was determined using linear regression analysis.

3. RESULTS AND DISCUSSION

The results of the total phenol and flavonoid content of the Avocado pear seed and leaf are presented in Table 1. The phenolic content of the leaf (92.85 mg/100 g) was significantly (P =.05) higher than the seed (57.1 mg/100 g); also, the flavonoid content of the leaf (50.95 mg/100g) was significantly (P =.05) higher than that of the seed (19.45 mg/100 g). However, the values obtained are lower than what was reported for some hot peppers [4], green teas [5] and some tropical leafy vegetables [28]. 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 antioxidant enzymes, reduce a-tocopherol radicals and inhibit oxidases [18]. Previous studies indicate that the aqueous leaf and seed extract of *P. americana* possessed cardio depressant, vasorelaxant and hypotensive (antihypertensive) effects in the experimental animal paradigms used [29]. Their potent antioxidant activity is due to the redox properties of their hydroxyl groups [30-32]. The presence of derivatives of flavonoids have been found in many plants; 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 [33,4]. Flavonoids have antioxidant activity and could therefore lower cellular oxidative stress [4]. Polyphenols are considered to be strong antioxidants due to the redox properties of their hydroxyl groups [30].

Table 1. Total phenol and flavonoid content of aqueous extract of avocado pear leaf and seed

	Phenol content (mg/100g)	Flavonoid content (mg/100g)
Seed	57.1^{a}±8.44	19.45^{c}±0.30
Leaf	92.85^{b}±9.12	50.95^{d}±0.61

Values represent means ± standard deviation of triplicate readings; Values with the same superscript letter along the same column are not significantly different (P=.05)

The result of the phytochemicals screening of the Avocado pear seed and leaf is represented in Table 2. Both the leaf and seed contained flavonoids and terpenoids compounds. In addition to these, the leaf contained tannins and saponins while the seed contained alkaloids. Many plants are rich sources of phytochemicals, and intakes of these plant chemicals have protective potential against degenerative diseases [34]. These different phytochemicals have various protective and therapeutic effects essentially to prevent diseases and maintaining a state of well being.

Table 2. Phytochemical screening of aqueous extract of avocado pear leaf and seed

Phytochemical	Seed	Leaf
Saponins	-	+
Alkaloids	+	-
Tannins	-	+
Anthraquinones	-	-
Flavonoid	+	+
Phlobatanins	-	-
Terpenoids	+	+

+ Present; - Absent

The ABTS* scavenging ability presented as trolox equivalent antioxidant capacity is presented in Fig. 1. The result revealed that both extracts can scavenge ABTS radical, however, the aqueous extract of the leaf (0.985 mmol. TEAC/g) had a significantly higher (P =.05) ABTS* scavenging ability than that of the seed (0.525 mmol. TEAC/g).

The ability of the water extractable phytochemicals from the Avovado pear leaf and seed to inhibit angiotensin -1- converting enzyme (ACE) activity *in vitro* was investigated and the result is presented in Fig. 2. The result revealed that both extracts inhibited angiotensin -1- converting enzyme (ACE) in a dose-dependent manner (in the range of 0–2.5 mg·ml-1), however, as revealed by the EC_{50} (extract concentration causing 50% enzyme inhibition) values (Table 3), seed (EC_{50} = 1.03 mg/ml) had a higher inhibitory activity than leaf (EC_{50} = 1.57 mg/ml). Furthermore, the effect of combining the avocado pear leaf and seed extracts in different proportion on angiotensin -1 converting enzyme (ACE) activity *in vitro* was also investigated and the result is presented in Fig. 3. The result revealed that combined avocado pear extracts also inhibited ACE activities. However, extracts of avocado pear combination exhibited an additive inhibitory effect.

Table 3. EC_{50} values of angiotensin – 1-converting enzyme inhibitory activity of aqueous extract of avocado pear leaf and seed

Samples	EC_{50} (mg/ml) Angiotensin-1-converting enzyme Inhibitory activity
Leaf	1.57±0.07
Seed	1.03±0.04

Values represent mean ± standard deviation, number of samples n = 3

ACE cleaves angiotensin I to angiotensin II, a powerful vasoconstrictor that has been identified as a major factor in hypertension [35]. As a result, ACE inhibitors have been widely developed to prevent angiotensin II production in cardiovascular diseases, and utilized in clinical applications since the discovery of ACE inhibitors in snake venom [36]. Inhibition of angiotensin-1 converting enzyme has been suggested as a useful therapeutic approach in the management/prevention of hypertension. The determined angiotensin-1 converting enzyme inhibitory activity agreed with some earlier reports where plant phytochemicals from *Citrus medica* inhibited angiotensin-1 converting enzyme and plants extracts of *Ginkgo. biloba*, and *Salvia. lavandulaefolia*, showed a significant improvement in cognitive performance and memory [37].

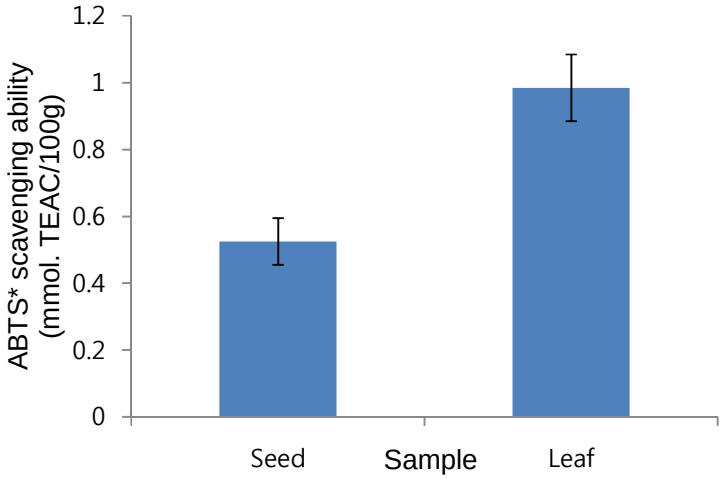

Fig. 1. ABTS radical scavenging ability of aqueous extract of avocado pear leaf and seed

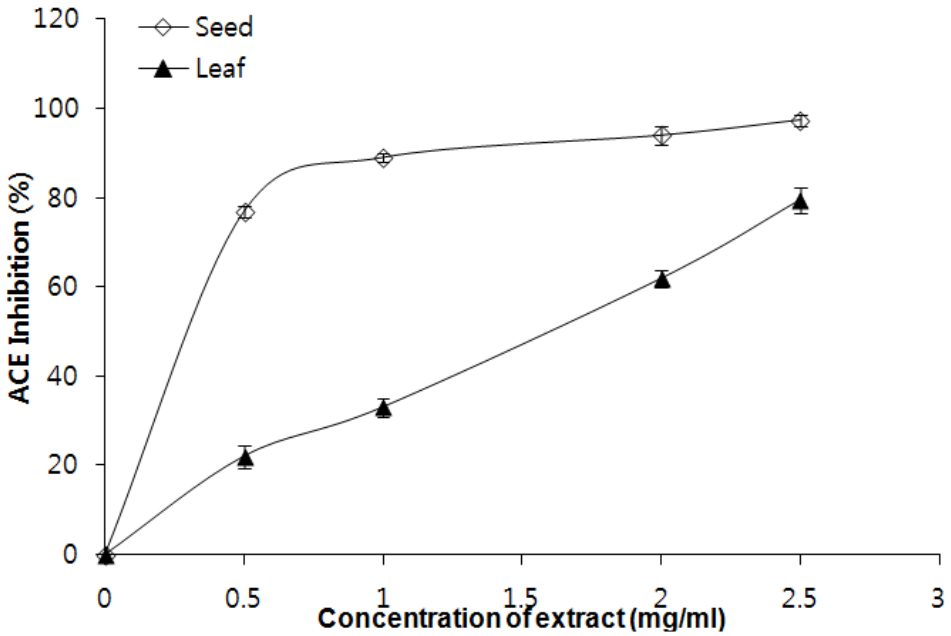

Fig. 2. Angiotensin-1-Converting enzyme inhibitory activity of aqueous extract of avocado pear leaf and seed

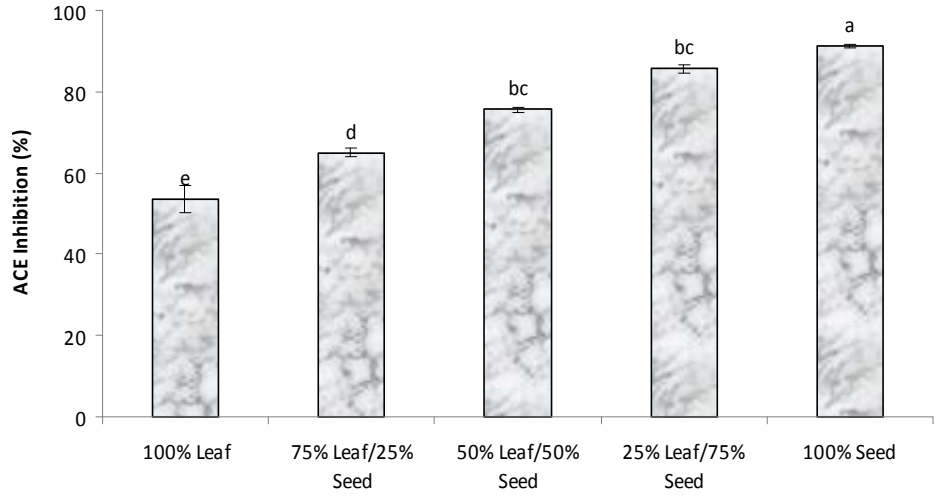

Fig. 3. Angiotensin-1-Converting enzyme inhibitory activity of aqueous extract of avocado pear leaf and seed mixture

4. CONCLUSION

The inhibitory effect of the Avocado pear leaf and seed could be due to some phytochemicals present in the extracts. The inhibition of enzyme linked with hypertension (angiotensin-1-converting activities) by aqueous extract of avocado pear leaf and seed could be part of the mechanism through which avocado pear leaf and seed manage and/ or prevent hypertension. However, the avocado pear seed was a better inhibitor of enzyme linked to hypertension (angiotensin-1-converting enzyme) than the avocado pear leaf.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX

CALCULATION

Total Phenol (mg/g)

Therefore, Absorbance of sample (Abssam) in mg/g will (Abssam * Conc. std) / (Absstd*Conc. sam)
Where, Absstd = Absorbance of Standard (Gallic acid)
Abssam = Absorbance of Sample
Conc. std = Stock Concentration of Standard in mg/ml
Conc. sam = Stock Concentration of Sample in g/ml

Total flavonoid (mg/g)

Therefore, Absorbance of sample (Abssam) in mg/g will (Abssam * Conc. std) / (Absstd*Conc. sam)
Where, Absstd = Absorbance of Standard (Quercetin)
Abssam = Absorbance of Sample
Conc. std = Stock Concentration of Standard in mg/ml
Conc. sam = Stock Concentration of Sample in g/ml

ABTS* scavenging ability (mmol. TEAC/g)

% scavenging ability = (Absref – Abssam) / Absref *100
Where, Absref = Absorbance of Reference
Abssam = Absorbance of Sample/Standard
Therefore, Percentage scavenging ability of sample (Persam) in mmol/g will be
(Persam * Conc. std) / (Perstd*Conc. sam * TMW)
Where, Perstd = Percentage scavenging ability of Standard (Trolox)
Persam = Percentage scavenging ability of Sample
Conc. std = Stock Concentration of Standard in mg/ml
Conc. sam = Stock Concentration of Sample in g/ml
TMW = Molecular mass of Trolox (264.32 g/mol)

Enzyme Inhibition (%)

% Inhibition = (Absref – Abssam) / Absref *100
Where, Absref = Absorbance of Reference
Abssam = Absorbance of Sample

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