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# Mechanisms of Anti-Hypertensive Activity of Methanol Leaf Extract and Fractions of *Persea americana* Mill. (Lauraceae) in Rats

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

## Abstract

**Background**: *Persea americana* has been identified to possess antihypertensive properties, but the mechanisms of these properties remain unclear. This study was thus designed to elucidate the mechanisms of anti-hypertensive activity of the extract and fractions of fresh leaf of *Persea americana* on hypertension induced in rats.

**Methods**: Crude methanol leaf extract of *P. americana* was partitioned into n-butanol, dichloromethane, chloroform, hexane and ethyl acetate. The ethyl acetate fraction was further purified through column chromatography. Doses of the extract and column fraction were tested for contraction on rat isolated thoracic rings and for *in vitro* angiotensin converting enzyme inhibitory activities. The test doses were further evaluated for anti-hypertensive activities on noradrenaline- and L-NAME-induced hypertension as well as atherogenic index in hyperlipidemic rats. Effects were compared with control values via one-way analysis of variance using GraphPad® version 8.0 software

**Results**: *P. americana* leaf methanol extract and ethyl acetate column fraction 3 reduced the contractile effect of noradrenaline significantly ($p < 0.001$) in the isolated thoracic aortic rings through intact endothelium-mediated mechanism(s). The test doses also exhibited *in vitro* angiotensin converting enzyme inhibitory activities similar to that of captopril. Additionally, ethyl acetate column fraction 3 also reduced the hypertensive effect of L-NAME- induced hypertension by 23%

**Conclusion**: *Persea americana* methanol leaf extract and ethyl acetate column fraction 3 exhibited significant reductions in all the tested parameters of L-NAME and noradrenaline-induced hypertension most probably through inhibition of vascular alpha adrenoceptors, angiotensin converting enzyme and amelioration of dyslipidemia.

***Keywords**: Persea americana*, Atherogenic index, Hypertension, Adrenoceptors, Angiotensin converting enzyme inhibitor

## INTRODUCTION

The prevalence of hypertension has thrown up a number of challenges including the need to have a better understanding of the role of the determinants of blood pressure so as to propose remedies for the condition right from this early stage. Though the mechanisms contributing to the cardiovascular disease progression are yet to be fully understood, nevertheless new therapeutic approaches are required (Gao *et. al*., 2020). Still, an additional need is to define the exact mechanisms of action of the chemical constituents of these new remedies so as to clearly understand their properties, focus on ways of synthesizing them in the future and predicting remedy for their possible side effects.

To date, much of our understanding of hypertension is centred on basic and preclinical research, in which animal models have been vital as a critical tool in advancing remedies for effective management (Park *et al*., 2019). Rodents (rats and mice) have been the most frequently used animals for these purposes for the following reasons: first, rodents are easier to maintain and also less costly compared to larger animal species, next, mice and rats reproduce quickly with a short lifespan and most importantly, the vast majority of insight into molecular and genetic mechanisms comes from genetically engineered mouse models (Le Bras, 2019).

All these advantages notwithstanding, many studies using rodents have resulted in conflicting and contradictory outcomes in clinical trials due to the major differences between rodents and humans, and only about a third of potential cellular targets identified in mouse models can be reproduced (Arrowsmith, 2011; Mullard, 2011), yet many more exciting therapeutic interventions are still being discovered and developed in mice and rats.

Ethnomedicine has offered a number of remedies for hypertension from a vast store of plants used in traditional medicine (Baharvand-Ahmadi, *et al.*, 2016; Malik, *et al.*, 2018). One of the plants that have shown prominent usefulness in managing hypertension is *Persea americana*, a perennial tree of the family Lauraceae (Dzuefiet, *et al.*, 2014; Sokpe *et al*., 2020) which is known to possess antihypertensive activities. The anti-hypertensive activities of *P. americana* are said to be due to a number of phytochemicals but the exact mechanism of these activities are still not clear.

This study, therefore, aimed at elucidating the mechanism(s) of action of the antihypertensive properties of the methanol leaf extract and fractions from *P. americana.* The leaves of *P. americana* were extracted in methanol and partitioned with *n*-hexane, dichloromethane, chloroform, ethyl acetate and *n*-butanol.

## METHODOLOGY

*Plant Collection*

The leaves of *Persea americana* Mill. (Lauraceae) were collected from the Department of Pharmacology, University of Ibadan, Ibadan. The plant was identified and authenticated by Mr. Donatus Eseimokhuai, Curator, University Herbarium as those of *P. americana* Mill. (family: Lauraceae) with identification number UIH2238, and a voucher specimen has been deposited in Department of Botany Herbarium, University of Ibadan.

### Extraction procedure of plant material

The leaves of *P. americana* were air-dried and milled in a Waring commercial blender. One and a half (1.5) kg of the powdered leaf was macerated in methanol/distilled water (95%/5%) and extracted twice, on each occasion with 3.5 L of the solvent at room temperature for 48 hours, with occasional shaking. The extracts were pooled together and concentrated to dryness at 40 ±1°C in Buchi Labortechnik® rotary evaporator Model CH-9230 (Switzerland) under reduced pressure to finally give 158.5 g (10.6 % yield) of a light-brown, powdery leaf extract. This *P. americana* methanol extract (PAM) was then partitioned into n-hexane, dichloromethane, chloroform, ethyl acetate and n-butanol fractions. Aliquot portions from the methanol extract and the different fractions were weighed and dissolved in physiologic saline for use on each day of the experiments in studying the possible mechanisms of antihypertensive action of the extract and fractions from *P. americana* leaf. The ethyl acetate fraction which is the most active of the fractions was further purified by column fractionation using isocratic solution.

### Effect of column fraction ethyl acetate (CFEt3) on rat isolated thoracic rings

The rats were euthanized with pentobarbital 120 mg/kg, the thoracic aorta was carefully extracted,

cleaned of extraneous tissue, sliced into rings of 3-4 mm in width and were suspended in a Krebs-Henseleit physiological solution organ bath kept at 36 $\pm 1^0$C and consistently bubbled with a mixture of carbon dioxide and oxygen (95% $O_2$ + $CO_2$). The tissue was allowed to equilibrate before being challenged with graded concentrations of noradrenaline, PAM 100mg/kg or CFEt3 25mg/kg. The contractile and/or relaxant effect of all applied test materials on the isolated rings were recorded by Powerlab®.

**Effect of the *in vitro* inhibitory activity of PAM and CFEt3 on angiotensin converting enzyme (ACE)**

The ACE inhibitory activity was estimated by a modification of the method of Vermeirssen, *et. al*, 2002. In this experiment, 50 µL of PAM or CFEt3 (12.5, 25, 50, 100, 200µg/mL) fraction (the inhibitor solution) and 50 µL of the enzyme were pre-incubated at 37 $^0$C for 10 min. The substrate - 200 µL Hippuryl-L-Histidyl-L-Leucine (HHL) was then added and the reaction was allowed to run for 90 min and later terminated by the addition of 250 µL 1N HCl. The hippuric acid (HA) formed was removed with 0.5 mL ethyl acetate and then centrifuged for 15 minutes at 3000 revolutions/min. From this, 0.2 mL ethyl acetate supernatant layer was put into a bottle. The hippuric acid formed was dissolved again in 1.0 mL distilled water and the absorbance was measured at 228 nm with Jenway 7315 (UK) ultraviolet/visible spectrophotometer. Percentage inhibition of angiotensin converting enzyme activity was determined using the formula:

% ACE inhibition = [B-A/B-C] x100,

Where A = Absorbance of HA generated in the presence of ACE inhibitor, B =Absorbance of HA generated without ACE inhibitor, C =Absorbance of HA generated without ACE, HA = Hippuric Acid. Captopril 100 µg/mL solution served as a positive control.

**Evaluation of the antihyperlipidemic effect of *P. americana* methanol leaf extract on Triton X-100-induced rats**

Thirty-five male Wistar rats (250-300g) were fasted overnight before the study but allowed clean water *ad libitum*. They were distributed into 7 groups (n=5) according to the modified method of Anyaegbu et al., 2017. Groups 1and 2; non-hyperlipidemic rats were treated orally with normal saline (10 mL/kg) and PAM 100mg/kg respectively. Rats in groups 3-7 were injected intraperitoneally with 10% solution of Triton X100 (100 mg/kg/day for 72h) to induce hyperlipidemia and subsequently treated orally for 7 days as follows: PAM (50 and 100 mg/kg), CFEt3 (25 mg/kg), atorvastatin (10 mg/kg) and normal saline (10 mL/kg). Rats body weights were recorded daily during the period of the experiment and the animals were anaesthetized afterwards with ether. Blood for serum lipid profile was collected through retro-orbital puncture into non-heparinized vacutainers and centrifuged at 4000 rpm for 10 min. The supernatant was removed for biochemical analysis. Serum total cholesterol (TC), triglyceride (TG) and high density lipoprotein cholesterol (HDL-C) were determined using Randox diagnostic kits (Randox Laboratories Ltd, United Kingdom). Low Density Lipoprotein-Cholesterol (LDL-C) and Very Low Density Lipoprotein were calculated using Friedewald's formula (Friedewald et al., 1972) as follows: VLDL = TG ÷ 5, LDL = Total Cholesterol (TC) – (VLDL + HDL). Atherogenic index was calculated by using the formula:

$$\text{Atherogenic Index (AI)} = \frac{\text{Total Cholesterol}}{\text{HDL Cholesterol}}$$

**Effect of the most active column fraction ethyl acetate (CFEt3) on L-NAME-induced increase in blood pressure.**

Hypertension was induced in rats through a 7-day intraperitoneal administration of L-NAME 80 mg/kg/day (Jaarin, 2015). Rats were weighed daily and at the end of induction of hypertension with L-NAME, rats were then treated with intraperitoneal injection of CFEt3, (25 mg/kg) for 7 days. Blood pressure and heart rate were measured using a computerised non-invasive system plethysmometer (model CODA®; 2096 Kent Scientific, Torrington, CT, USA). In this method, the rats were restrained on a preheated platform with the tail exposed. A cuff that occluded the rat tail vein and a cuff for recording volume pressure were fixed on the tail. Afterwards, twenty (20) cycles of recordings were made for each rat to obtain rat blood pressure. Care was taken to ensure that the rats were stable before commencing the recordings which were made by the same person under similar environmental conditions.

**Determination of the phytochemical constituents of the most active pooled column fraction using Gas Chromatography-Mass Spectrometry (GC-MS)**

The ethyl acetate fraction of the methanol leaf extract of *P. americana* was analysed using GC-MS Agilent Technologies 7890 system mass spectrometric detector (Agilent technologies 5975 model). The GC analysis is a separation technique where there are two

phases – the mobile phase and the stationary phase. The mobile phase is the carrier gas (Helium, 99.99% purity), while the stationary phase is the column. The column is of length 30 m, internal diameter 0.320 mm, while the thickness is 0.25 µm (Thomas *et al*., 2013). The oven was programmed at an initial temperature of 80 $^0$C to hold for 1 minute and to increase by 100$^0$C per minute up to the final temperature of 240 $^0$C to last for 6 minutes. The fraction was injected at a volume of 1µL and detector temperature of 250$^0$C. The ionization voltage was 70eV and the mass spectral scan range was 45-500 MHz.

**Statistical Analysis**

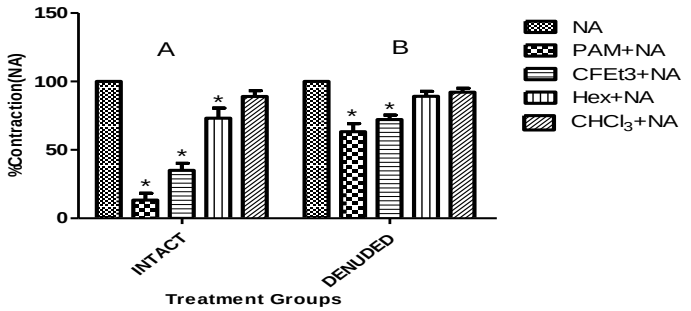
Data were expressed as Mean ± Standard Error of Mean. Some outcomes were examined using Student t-test. Test doses were compared with control values via one-way analysis of variance (ANOVA) and post-hoc Dunnett's Multiple Comparison Test. P-values less than 0.05 ($p < 0.05$) were considered statistically significant while analysis was performed using GraphPad® prism version 8.0 software.

## RESULTS

**Percentage response of endothelium-intact and endothelium-denuded aorta to noradrenaline in the presence of *Persea americana* methanol leaf extract and fractions**

Noradrenaline $10^{-5}$M contracted the endothelium intact aorta maximally (100%). The contraction, (Fig 1a) produced by noradrenaline in the presence of *Persea americana* leaf methanol extract 2 mg/ml (PAM), ethyl acetate column fraction 3, 2 mg/ml (CFEt3), hexane fraction 2 mg/ml and chloroform fraction 2 mg/ml were 13%, 35%, 73% and 89% respectively, while , in the endothelium denuded aortic ring, the contractions were as follows: *P. americana* leaf methanol extract (63.4%), column fraction of ethyl acetate 3 (72.2%), hexane fraction (89.2%) and chloroform fraction (91.5%). The result of percentage reduction in contraction of endothelium-intact and endothelium-denuded aorta to noradrenaline in the presence of *Persea americana* methanol leaf extract and fractions (2 mg/ml) are presented in figure 1b. The results obtained for endothelium-intact aortic rings are as follows: noradrenaline $10^{-5}$M (0%), *Persea americana* methanol leaf extract (74.4%), column fraction of ethyl acetate fraction 3, 2 mg/ml (65%), hexane fraction 2 mg/ml (27.8%) and chloroform fraction 2 mg/ml (11%). The values for percentage reduction in contraction for endothelium denuded aorta are also as follows: noradrenaline (0%), *Persea americana* methanol leaf extract (36.6%), column fraction of ethyl acetate fraction 3 (27.8%), hexane fraction (10.8%) and chloroform (8.5%). Thus *P. americana* leaf methanol extract, ethyl acetate column fraction 3 and hexane fraction reduced the contractile effect of noradrenaline significantly in the endothelium intact aorta. This indicates that the endothelium is necessary for the activity of the extract and fractions.

**(a)**




**(b)**

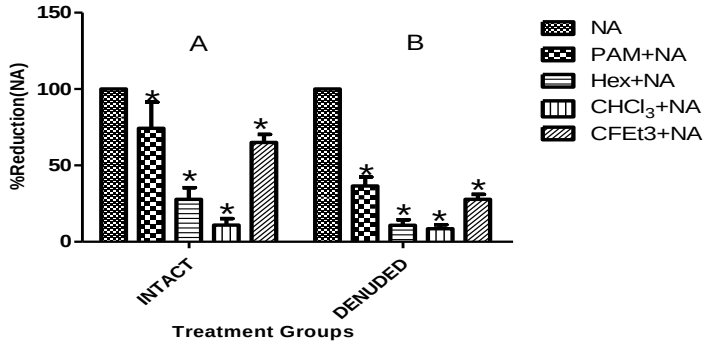



**Fig. 1: Percentage response of endothelium intact and endothelium denuded aorta to noradrenaline in the presence of *Persea americana* methanol leaf extract and fractions; (a) % Contraction (b) % Reduction**

NA- Noradrenaline ($10^{-5}$ M), PAM- *P. americana* leaf methanol extract 2 mg/ml, Hex- Hexane fraction 2 mg/ml, $CHCl_3$- Chloroform fraction 2 mg/ml, CFEt3- Column fraction of ethyl acetate fraction 3 (2 mg/ml). Values represent the mean ± S.E.M of 5 aortic rings per group. ANOVA followed by Dunnett's post hoc test. * $p < 0.05$.

**Inhibition of angiotensin converting enzyme by *P. americana* methanol leaf extract and ethyl acetate column fraction 3**

The *in vitro* angiotensin converting enzyme inhibitory activities of *Persea americana* methanol leaf extract and column fraction of ethyl acetate fraction 3 were matched with captopril. From available results (fig 2a), activity of both extract and fraction are similar. However, the purer column fraction may possess the advantage of being devoid of the components that may possibly cause adverse effects in the crude extract. In figure 2b, the result of the percentage inhibition of ACE by *P. americana* methanol leaf extract, ethyl acetate column fraction 3 and captopril (100 µg/ml each) revealed that the extract and CFEt3 inhibited angiotensin converting enzyme by 70% and 73. 6% respectively compared with that of captopril (68.1%). Also $IC_{50}$ of PAM was 76.24 ± 0.09 µg/mL, CFEt3 was 72.51 ± 0.01 µg/mL, while that of captopril was 61.92 ± 0.02 µg/mL

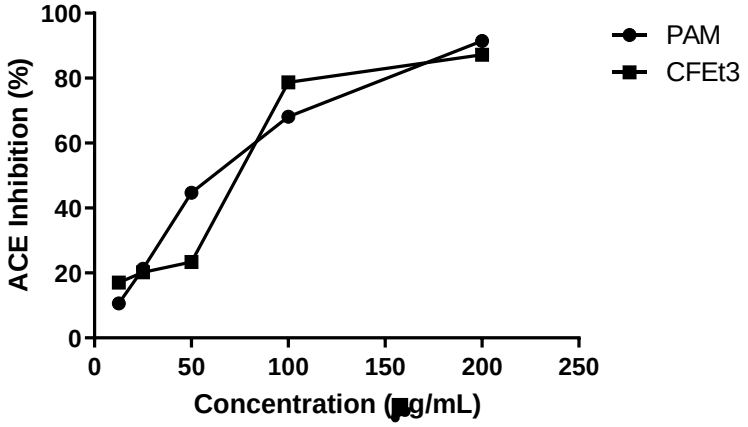



**Fig. 2a: Percentage angiotensin converting enzyme inhibition by *P. americana* crude leaf extract and ethyl acetate column fraction 3**

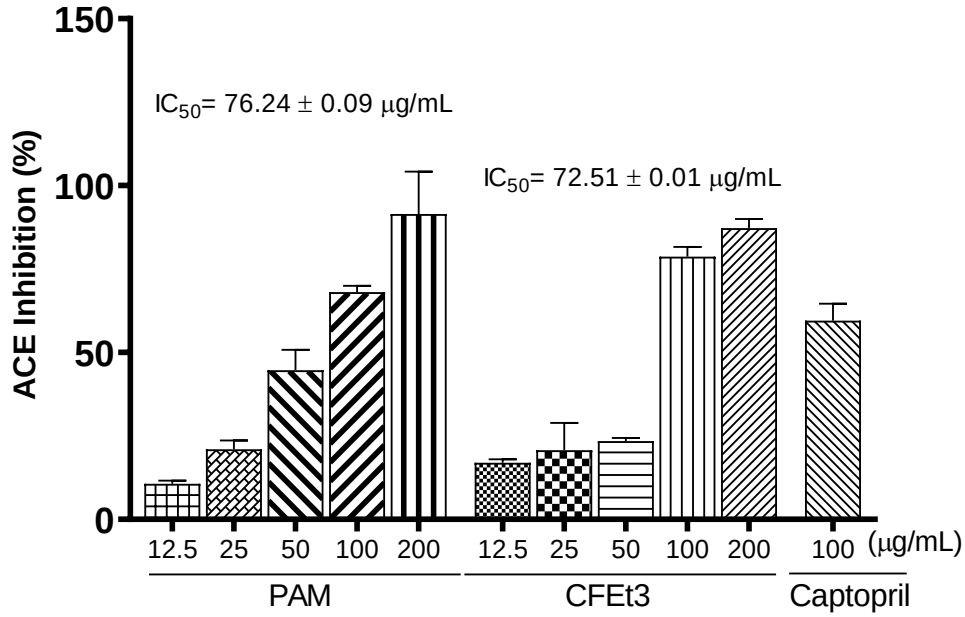



**Fig. 2b: Percentage angiotensin converting enzyme inhibition by *P. americana* methanol leaf extract, column fraction of ethyl acetate and captopril**

PAM- *P. americana* methanol leaf extract, CFEt3- ethyl acetate column fraction 3.

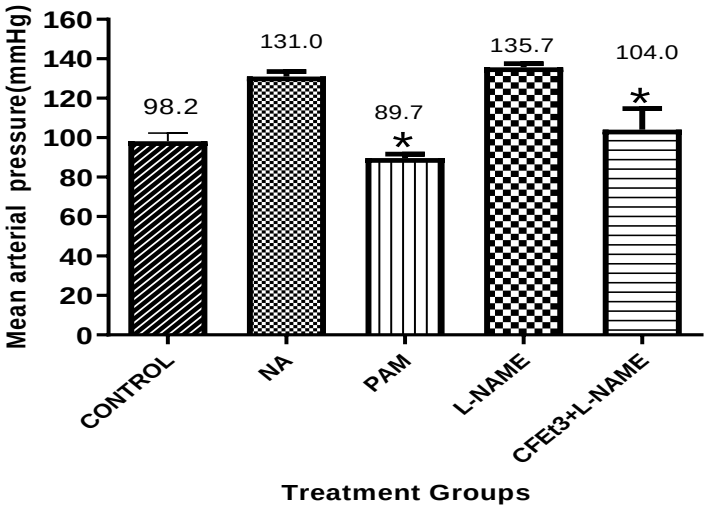
**Table 1: Effect of oral administration of *Persea americana* methanol leaf extract and CFEt3 on lipid profile when hyperlipidemia was induced in rats using Triton X-100**

| Treatment | TCHOL (mg/dL) | TRIG (mg/dL) | HDL (mg/dL) | LDL (mg/dL) | VLDL (mg/dL) | AI |
|---|---|---|---|---|---|---|
| Normal control | 89.58 ± 7.69 | 115.46 ± 2.84 | 62.30 ± 4.01 | 4.19 ± 4.37 | 23.09 ± 1.01 | 0.43 ± 0.03 |
| TX- 100 | 150.94 ± 5.92# | 144.60 ± 2.66 | 56.12 ± 7.28 | 65.89 ± 20.96# | 28.92 ± 0.62 | 1.81 ± 0.51 |
| PAM 100 | 87.11 ± 4.49 | 63.10 ± 1.40* | 127. 67 ± 6.83* | -22.53 ± 15.86* | 16.25 ± 1.02* | 0.03 ± 0.12* |
| TX +AT 10 | 126.89 ± 9.82 | 142.70 ± 12.82 | 94.72 ± 3.28* | 3.63 ± 27.84* | 28.54 ± 2.56 | 0.03 ± 0.001* |
| TX+PAM 50 | 149.467 ± 5.61 | 141.68 ± 3.07 | 97.58 ± 8.16* | 23.55 ± 21.90* | 28.34 ± 0.61 | 0.57 ± 0.28 |
| TX+ PAM 100 | 115.30 ± 8.64* | 81.23 ± 0.66* | 121.59 ± 5.38* | -22.53 ± 15.86* | 16.25 ± 1.02 | 0.03 ± 0.001* |
| TX + CFEt3 | 87.13 ± 4.49* | 63.11 ± 1.40* | 125. 67 ± 6.83* | -22.50 ± 15.86* | 15.25 ± 1.02* | 0.03 ± 0.01* |

TCHOL- Total Cholesterol, TRIG - Triglycerides, HDL- High Density Lipoprotein, LDL-Low Density Lipoprotein, VLDL- Very Low Density Lipoprotein, AI- Atherogenic Index, PAM 50,100- *P. americana* leaf methanol extract, 50 mg/kg, 100 mg/kg respectively; AT 10- Atorvastatin 10 mg/kg., CFEt3- ethyl acetate column fraction 3 25 mg/kg. # $p < 0.05$ vs NC, * $p < 0.05$ vs TX-100

**Effect of $N^G$-nitro-L-arginine methyl ester alone or with column fraction of ethyl acetate 3 on mean arterial pressure of rats**

In figure 6, noradrenaline (4.0 µg/kg) increased mean arterial pressure of tested rats from 98.4 ± 4.1 mmHg to a mean of 131.0 ± 2.5 mmHg, while $N^G$-nitro-L-arginine methyl ester **(**L-NAME) 80 mg/kg/day for 7 days raised mean arterial pressure to 135.7 ± 1.9 mmHg. However, when column fraction of ethyl acetate 3 25 mg/kg (CFEt3) was administered before L-NAME was injected, there was a significant 23.3% (104.0 ± 10.7 mmHg) reduction in mean arterial pressure previously raised by L-NAME. This indicates the inhibitory effect of column fraction of ethyl acetate 3 on the hypertensive effect of L-NAME.




NA- Noradrenaline 4.0 µg/kg, PAM 100 mg/kg, L-NAME-$N^G$-nitro-L-arginine methyl ester 80 mg/kg/day for 7 days, CFEt3- ethyl acetate column fraction 3 25 mg/kg. Values represent the mean ± S.E.M of 5 rats per group. ANOVA followed by Dunnett's post hoc test. * $p < 0.05$

**Fig. 6: Effect of $N^G$-nitro-L-arginine methyl ester or with ethyl acetate column fraction 3 on mean arterial pressure of rats**

**GMS Analysis**

The GCMS analysis identified 11 compounds as shown in Table 2. Of these compounds identified, 11-Tetradecyn-1-ol acetate (16.6%), 8-Hexadecenal,14-methyl-(Z)- (16.5%) and Cyclopropane carboxaldehyde (12.92%) were the most abundant components of the fraction.

**Table 2: identification of chemical components of ethyl acetate fraction of *Persea americana* using GC-MS**

| Peak No | Compound | GC-MS-RT (min) | Percentage of total/Abundance (%) | M+1 Value |
|---|---|---|---|---|
| 1 | 9-Tetradecen-1-ol,acetate | 21.864 | 8.196 | 252 |
| 2 | Cyclopropane carboxaldehyde | 22.023 | 12.198 | 70 |
| 3 | Bicyclo(3.1.1)heptane-2,3-diol, 2,6,6-trimethyl- | 22.041 | 2.146 | 170 |
| 4 | 1,14-Tetradecanediol | 22.059 | 1.762 | 230 |
| 5 | 8-Hexadecenal, 14-methyl-, (Z)- | 22.191 | 16.455 | 250 |
| 6 | 11-Hexadecen-1-ol, (Z)- | 22.213 | 3.216 | 240 |
| 7 | Chloroacetic acid, 10-undeceny ester | 22.236 | 4.870 | 246 |
| 8 | 7-Methyl-Z-8,10-dodecadienal | 22.301 | 9.048 | 170 |
| 9 | 11-Tetradecyn-1-ol acetate | 22.416 | 16.593 | 252 |
| 10 | 2,7-Octadiene, 4-methyl-` | 22.447 | 7.031 | 124 |
| 11 | Cyclohexene, 3-(2-methylpropoxy)- | 22.487 | 7.571 | 154 |

## DISCUSSION

In this study, in the rat thoracic aorta pre-contracted with noradrenaline $10^{-5}$M, ethyl acetate column fraction 3 offered significant ($p< 0.05$) reduction in the contraction of endothelium-intact aortic rings. The reduction in the contraction in endothelium-denuded aortic rings was however not significant. These findings indicate that the action of the ethyl acetate column fraction requires intact endothelium. Ojewole *et al*., (2007) in an earlier study had stated that endothelium-containing aortic rings pre-contracted with noradrenaline in Krebs-Henseleit solution with and without normal calcium concentrations were relaxed by exogenous additions of *P. americana* aqueous leaf extract or acetylcholine. They also proposed a mechanism of non-competitive $\alpha_1$-adrenoceptor blockade.

In the endothelium-stripped aortic rings, there is decrease in the bioavailability of nitric oxide (Jaarin *et al*., 2015), an endothelium-dependent relaxation factor that eases blood pressure homeostasis (Albrecht, 2003). Under standard physiological conditions, endothelial nitric oxide synthase produces basal levels of nitric oxide vascular stability. Nitric oxide promotes vascular smooth muscle relaxation by stimulating soluble guanylate cyclase and by augmenting cyclic guanosine - 3',5'- monophosphate (cGMP), which inactivates the release of intracellular calcium. Increases in intracellular calcium are associated with contraction of vascular smooth muscle. Prolonged vascular smooth muscle contraction is thought to start fundamental changes inside the vessel, for instance, thickening, which can initiate an irreversible increase in peripheral resistance. Furthermore, studies have shown that raised peripheral resistance is connected with primary hypertension (Munzel *et al*., 2003; Ko *et al*., 2013; Harraz *et al.,* 2014). As a consequence of its effect on smooth muscle relaxation, nitric oxide plays a central function in the physiological regulation of blood pressure and protecting the vasculature.

The arterial muscle relaxant effect of the extract disappeared on removal of the functional endothelium. This vasorelaxant property (endothelium) would appear to have contributed, at least in part, to the antihypertensive effect of the plant *P. americana.*

These observations therefore indicate that the vasorelaxant effects of *P. americana* and ethyl acetate column fraction 3 were dependent, in part, on the formation, synthesis and/or release of endothelium-derived nitric oxide, since removal of the functional endothelial cells led to the absence of relaxant response. The present study also suggests that the endothelium-dependent vasorelaxant effect of *Persea americana* methanol leaf extract and ethyl acetate column fraction 3 could be mediated via endothelial nitric oxide signalling in the aortic tissue preparations. Thus, maintaining intact endothelial integrity is necessary for normal blood pressure as distorted endothelium can facilitate vascular damage and hypertension.

Angiotensin II is a potent vasoconstrictor whose formation is catalysed by Angiotensin-converting enzyme (ACE). Inhibition of ACE therefore plays a major role in the control of blood pressure. In this study, the inhibitory effect of PAM and CFEt3 was determined by a modification of the method described by Vermiessen *et al*., (2002) and expressed as $IC_{50}$ - the concentration of sample needed to inhibit 50% of ACE activity.

ACE inhibition was measured using Hippuryl-L-Histidyl-L-Luecine as substrate, rabbit lung acetone extract as ACE source and Captopril as standard drug. This study further revealed that *Persea americana* methanol leaf extract and ethyl acetate column fraction 3 showed significant angiotensin converting enzyme inhibitory activity. The ability of the treatments to block this enzyme indicated that on administration to the anaesthetized rats, there will be less Angiotensin II formed and hence less vasoconstricting effect on blood vessels, leading to a decrease in blood pressure. An earlier report by Vermeirssen *et al*., (2002) had noted a comparable effect with Captopril. From this study, the $IC_{50}$ value of ACE inhibition assay of methanol leaf extract and ethyl acetate column fraction 3 from *P. americana* leaf and Captopril revealed that Captopril exhibited a superior, yet comparative inhibition over both extract and fraction ($p > 0.05$). The presence of enzyme inhibitory activity in *P. americana* methanol leaf extract and ethyl acetate column fraction 3 therefore, is a noteworthy addition to understanding the probable antihypertensive mechanism of *P. americana.* In addition, more natural, potent angiotensin converting enzyme inhibitors might well be isolated by further chemical purification and investigation from this fraction, or from other parts of the plant.

Hypertension has been associated with other cardiovascular risk factors, for example, obesity, diabetes, and dyslipidemia (Segura and Ruilope, 2007). Hyperlipidemia, a form of dyslipidemia, has been described as elevated plasma concentrations of lipids - triglycerides (TG) and total cholesterol (TC) - and their blood transporting lipoproteins; HDL-Cholesterol, LDL-Cholesterol and VLDL-Cholesterol (Nwagha, *et al*., 2010).

Additionally, several other researches have noted the joint occurrence of hypertension and dyslipidemia in the same patients (Thomas and Bean, 2002; Wong *et al*, 2006). Moreover, higher incidences of hypertension usually follow elevated values of Low Density Lipoprotein-Cholesterol, whereas increased

High Density Lipoprotein-Cholesterol (HDL-C) values have decreased episodes of hypertension (Nwagha, *et al*., 2010) Because elevated lipid levels have been found to predate the onset of hypertension, it has thus been suggested that lipids may offer a potentially important screening tool with which to identify high risk individuals for hypertension over time and therefore, proper handling of dyslipidemia will have a positive outcome on blood pressure (Dobiasova and Frohlich, 2001; Halperin, 2006; Ford, 2011).

The atherogenic index of plasma characterized as logarithm [log] of the proportion of plasma concentration of triglycerides to high-density lipoprotein (HDL) cholesterol, has lately been proposed as an insightful marker for plasma atherogenicity and is unequivocally related with cardiovascular disease risk (Dobiasova, *et al*., 2001; George, 2006). The atherogenic index of plasma (AIP) reflects the genuine connection amongst protective and atherogenic lipoprotein and is related with the level of pro- and anti- atherogenic lipoprotein molecule. It has been proposed that an AIP estimation of under 0.11 is related with generally low incidence of cardiovascular diseases and greater values predict higher incidence (Dobiasova, *et al., 2011*).

In this study. the antilipidemic activities of the extract and fraction were tested on Triton X-100 - induced hyperlipidemic rats and the results showed that *P. americana* methanol leaf extract and ethyl acetate column fraction 3 demonstrated positive values of atherogenic index of the plasma by reducing the levels of total cholesterol and triglycerides with a corresponding elevation in the values of high density lipoprotein. The Artherogenic Index of Plasma for both *Persea americana* methanol leaf extract and ethyl acetate column fraction 3 was 0.03 ± 0.001. This is similar to the Artherogenic Index of Plasma for Atorvastatin, a standard statin lipid lowering drug (Hannan *et al*., 2016). This lipid lowering effect may offer a protection against the damage of vascular endothelium that is common in hypertension resulting from hyperlipidemia

Also, in this study, the intraperitoneal administration of $N^G$-Nitro-L-arginine methyl ester (L-NAME), a nitric oxide synthase inhibitor increased rodent blood pressure. This is in agreement with the discoveries of prior investigations by Beratova *et al*., (1999) who had earlier reported similar observations. However, intravenous administration of *P. americana* methanol leaf extract and its ethyl acetate column fraction 3 significantly reduced L-NAME-induced raised systolic blood pressure, diastolic blood pressure and mean arterial pressure. This effectively prevented the dysfunction in the synthesis of nitric oxide caused by L-NAME and therefore the imbalance between the vasoconstricting and vasorelaxing factors which is a common feature in several models of hypertension. This observation was in concurrence with different investigations by El Tahir *et al*., (2003), Khattab *et al*., (2007) and Sayed *et al*., (2009). Nevertheless, these treatments had no impact on L-NAME-induced increase in heart rate.

It is noted that some previous studies have identified flavonoids and tannins; terpenoids and saponins (Adeyemi, *et al*., 2002), as constituents of the leaf of *P. americana*. That the additionally identified compounds from ethyl acetate column fraction 3 by GC-MS still relaxed aortic rings pre-contracted with noradrenaline and L-NAME, blocked angiotensin converting enzyme *in vitro* and reduced hyperlipidemia in experimental animal models is a strong indication of the antihypertensive effects of the leaf of *P. americana*. It also indicates that the leaf of the plant contains compounds that may be of use in the development of antihypertensive drugs. The most abundant components of column fraction ethyl acetate 3 were 11-Tetradecyn-1-ol acetate and 8-Hexadecenal, 14-methyl-, (Z). It is most probable that these constituents contributed in no small way to the observed anihypertensive effect of the fraction.

## CONCLUSION

In conclusion, the vasorelaxant and antihypertensive effects of *Persea american* exhibited in this study may be mediated through inhibition of vascular alpha adrenoceptors, angiotensin converting enzyme and amelioration of dyslipidemia.

## REFERENCES

Adeyemi, O. O., Okpo, S.O and Ogunti, O. 2002. Analgesic and anti-inflammatory effects of the aqueous extract of leaves of *Persea americana* Mill (Lauraceae). *Fitoterapia* 73: 375-380.

Albrecht, E. W. J. A., Stegeman, C. A., Heeringa, P., Henning, R. H and van Goor, H. 2003. Protective role of endothelial nitric oxide synthase. *Journal of Pathology* 199(1): 8-17.

Arrowsmith J. 2011. Phase II failures: 2008–2010. *Nature Reviews Drug Discovery10*:328–9.

Baharvand-Ahmai B, Bahmani M, Tajeddini P, Rafieian-Kopaei M and Naghdi N. 2016. An ethnobotanical study of medicinal plants administered for the treatment of hypertension. *Journal of Injury Prevention.* 5(3): 123-128

Beratova, I., Pechanova, O. G and Simko, F. 1999. Effect of captopril in L-NAME induced hypertension on rat's myocardium, aorta, brain and kidney, *Experimental Physiology* 84(6):1095-105 http://dx.doi.org/10.1111/j.1469-445X.1999.01890.

Weil, B. R., Stauffer, B. L., Greiner, J. J and DeSouza, C. A. 2011. Prehypertension Is Associated With Impaired Nitric Oxide-Mediated Endothelium-Dependent Vasodilation in Sedentary Adults. *American Journal of Hypertension*, Vol. 24; 9:976–981 https://doi.org/10.1038/ajh.2011.88

Dzeufiet, P. D. D., Mogueo, A., Bilanda, D. C., Aboubakar, B. O., Tedong, L., Dimo T and Kamtchouing P. 2014. Antihypertensive potential of the aqueous extract which combine leaf of Persea americana Mill. (Lauraceae), stems and leaf of Cymbopogon citratus (D.C) Stapf. (Poaceae), fruits of Citrus medical L. (Rutaceae) as well as honey in ethanol and sucrose experimental model. BMC Complementary and Alternative Medicine 14:507 http://www.biomedcentral.com/1472-6882/14/507

Dobiasova, M and Frohlich, J. 2001. The plasma parameter log (TG/HDL-C) as an atherogenic index: Correlation with lipoprotein particle size and esterification rate in apoB-lipoprotein-depleted plasma (FER (HDL), *Clinical Biochemistry* 43(7) 583-558.

Dzeufiet, P. D. D., Mogueo, A., Bilanda, D. C., Aboubakar, B.-F. O., Tédong, L., Dimo, T., and Kamtchouing, P. 2014. Antihypertensive potential of the aqueous extract which combine leaf of *Persea americana* Mill. (Lauraceae), stems and leaf of *Cymbopogon citratus* (D.C) Stapf. (Poaceae), fruits of *Citrus medical* L. (Rutaceae) as well as honey in ethanol and sucrose experimental model. BMC Complementary and Alternative Medicine, *14(1).* doi:10.1186/1472-6882-14-507

El Tahir, K. E. H., Al Ajmiand, M. F and Al-Bekari, A. M. 2003. Some cardiovascular effects of dethymoquinonated *Nigella sativa* volatile oil and its major components a-pinene and p-cymene in rats. *Saudi Pharmaceutical Journal* 11(3):104-10.

Ford, E. S. 2011. Trends in mortality from all causes and cardiovascular disease among hypertensive and nonhypertensive adults in the United States. *Circulation* 123(16):1737-44.

Friedewald, W. T., Levy, R. I and Fredrickson, D. S. 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. Jun;18 (6):499-502. PMID: 4337382.

George, E. 2006. Differential effect of hormone therapy and tibolone on lipids, lipoproteins, and the atherogenic index of plasma. *Journal of Cardiovascular Pharmacology* 47(4) 542-548.

Gao, Q., Xu, L and Cai, J 2020. New drug targets for hypertension: A literature review, BBA - Molecular Basis of Disease https://doi.org/10.1016/ j.bbadis.2020.166037

Halperin, R. O., Sesso, H. D and Ma, J. 2006. Dyslipidemia and the risk of incident hypertension in men. *Hypertension* 47:45-50.

Harraz, O. F., Brett, S. E. and Welsh, D. G. 2014. Nitric oxide suppresses vascular voltage-gated T-type Ca2+ channels through cGMP/PKG signaling. *American Journal of Physiology-Heart and Circulation Physiology* 306: H279-85.

Jaarin, K., Foong, W. D., Yeoh, M. H., Kamarul, Z. Y., Qodriyah, H. M and Azman A. 2015. Mechanisms of the antihypertensive effects of *Nigella sativa* oil in L-NAME-induced hypertensive rats. *Clinics* 70(11):751-757.

Khattab, M. M and Nagi, M. N. 2007. Thymoquinone supplementation attenuates hypertension and renal damage in nitric oxide deficient hypertensive rats. *Phytotherapy Research* 21(5):410-4.

Ko, M. L., Shi, L., Huang, C. C, Grushin, K., Park, S. Y and Ko, G. Y. 2013. Circadian phase-dependent effect of nitric oxide on L-type voltage-gated calcium channels in avian cone photoreceptors. *Journal of Neurochemistry* 127: 314-28.

Le Bras, A. 2019. A resource for selecting animal models of heart disease. Lab Animal, 48(11), pp.332-332. https://www.nature.com/articles/s41684-019-0425-4

Malik K, Ahmad M, Bussmann R. W., Tariq A, Ullah R, Alqahtani A. S., Shahat A. A., Rashid N, Zafar M, Sultana S and Shah S. N. 2018. Ethnobotany of Anti-hypertensive Plants Used in Northern Pakistan. Frontiers in Pharmacology 9:789

Mullard A. 2011. Reliability of "new drug target" claims called into question. *Nature Reviews Drug Discovery* 10:643–4

Munzel, T., Feil, R., Mulsch, A., Lohmann, S. M., Hofmann, F and Walter U. 2003. Physiology and pathophysiology of vascular signalling controlled by guanosine 3',5'-cyclic monophosphate-dependent protein kinase. *Circulation* 108: 2172-83.

Nwagha, U. I., Ikekpeazu, E. J., Ejezie, F. E., Neboh. E. E and Maduka, I. C. 2010. Artherogenic index of plasma as useful predictor of cardiovascular risk among postmenopausal women in Enugu, Nigeria. *African Health Sciences* 10: 248-252

Ojewole, J. A., Kamadyaapa, D. R., Gondwe, M. M., Moodley K and Musabayane C. T. 2007. Cardiovascular effects of *Persea americana* Mill (Laureceae) (avocado) aqueous leaf extract in experimental animals *Cardiovascular Journal of Africa* 18: 69-76.

Park, S. E and Schaer, T. P. 2019 "Preclinical Animal Models," Academic Entrepreneurship for Medical and Health Scientists: Vol. 1 : Iss. 3 , Article 20.

Sayed, H. M., El-Latif, H. A. A., Eid, N. I., Elsayed, A. Z and El-Kader, E. M. A. 2009. Potential antihypertensive and antioxidative effects of *Nigella sativa* seeds or biomass and Syzygium aromaticum extracts on L-NAME-induced hypertensive rats. *Egyptian Journal of Pharmaceutical Sciences* 50:127-46.

Segura, J and Ruilope, L. M. 2007. Obesity, essential hypertension and renin-angiotensin system. *Public Health Nutrition* 10:1151-5.

Sokpe, A., Merlin L. K. Mensah, M. L. K., Koffuor, G. A., Thomford, K. P., Arthur, R., Jibira, Y., Baah, M. K., Adedi, B. and Agbemenyah, H. Y. 2020. "Hypotensive and Antihypertensive Properties and Safety for Use of *Annona muricata* and *Persea americana* and Their Combination Products", *Evidence-Based Complementary and Alternative Medicine*, vol. 2020, Article ID 8833828, 13 pages. https://doi.org/10.1155/2020/8833828

Thomas, E., Aneesh, T. P., Thomas, D. G and Anandan, R. 2013. GC-MS Analysis of Phytochemical Compounds Present In The Rhizomes of *Nervilia aragoana* Gaud., *Asian Journal Of Pharmaceutical And Clinical Research*, Vol 6, Suppl. 3, 0974-2441.

Thomas, F and Bean K. 2002. Combined effects of systolic blood pressure and serum cholesterol on cardiovascular mortality in young (<55years) men and women. *European Heart Journal* 23: 528-535.

Vermeirssen, V. Camp J. V. Verstra ete, W. 2002. Optimization and validation of an angiotensin-converting enzyme inhibition assay for the screening of bioactive peptides. *J. Biochem. Biophys*. *Methods* 51 (2002) 75-87.

Wong, N. D., Lopez, V and Tang S. 2006. Prevalence, treatment, and control of combined hypertension and hypercholesterolemia in the United States. *American Journal of Cardiology* 98:204–208.

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