

***Persea americana* Leaf Extracts Demonstrates Enviably In vitro Antioxidant, Anti-inflammatory and Antihypertensive Properties**

¹Adejumobi O. A., ²Ekundayo S., ¹Omotosho O.O., ⁶Gbadegoye J.O., ¹Ajani T.F.,
⁵Balogun O.F., ²Oyagbemi A.A., ³Adedapo A.A., ⁴Yakubu M.A., ⁵Ashafa A.O.T.,
¹Nottidge H.O. and ¹Omobowale T.O.

¹Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Ibadan.

²Department of Veterinary Physiology and Biochemistry, University of Ibadan, Nigeria.

³Toxicology and Assessment Section, Michigan Department of Health and Human Services, Lansing, MI, USA

⁴Department of Environmental and Interdisciplinary Studies, Center for Cardiovascular Diseases, COPHS, Texas State University, Houston, TX, U.S.A.

⁵Department of Plant Science, University of Free State, Qwaqwa Campus, South Africa.

⁶Department of Physiology, University of Tennessee Health Sciences Centre, USA.

*Corresponding author: muyenko@yahoo.com Tel: +2348033728003

Abstract

Scientific validation is crucial to substantiate therapeutic claims associated with natural products. This study examined the in vitro antioxidant, anti-inflammatory, and antihypertensive activities of *Persea americana* (PA) leaf extract which is an ethnomedicinal plant used in disease management in Africa.

Following defatting with n-hexane, plant material was extracted using methanol and evaporated using rotary evaporator. The extract was then partitioned into n-hexane, chloroform, ethyl acetate, n-butanol, and aqueous-methanol fractions. Antioxidant assays included DPPH, nitric oxide, ABTS radical scavenging, metal chelation, reducing power, and hydroxyl radical inhibition. Anti-inflammatory activity was assessed via 15-lipoxygenase inhibition, while antihypertensive potential was measured through angiotensin I-converting enzyme (ACE) inhibition. Absorbance was measured using Elisa microplate reader and IC₅₀ determined by using IC calibration curve. Statistical analysis was done by ANOVA

The n-butanol fraction showed the most potent ABTS scavenging activity (IC₅₀ = 12.93 ± 1.10 µg/mL), outperforming both the crude extract and standard, gallic acid. This fraction also exhibited the strongest DPPH inhibition (IC₅₀ = 24.67 ± 5.13 µg/mL). The aqueous-methanol fraction was most effective against hydroxyl radicals, while metal chelating and nitric oxide inhibitory activities were highest in the chloroform and n-hexane fractions, respectively. All fractions demonstrated maximal reducing power at 250 µg/mL, except for the n-butanol fraction at 125 µg/mL. Notably, the n-butanol fraction inhibited 15-lipoxygenase more effectively than indomethacin, though less than quercetin. However, the crude extract's ACE inhibition was significantly lower than that of captopril.

These findings suggest that *Persea americana* leaf fractions exhibit diverse in vitro bioactivities and may offer therapeutic benefits in managing hypertension and inflammation-related disorders. However, the precise mechanisms underlying these effects remain unclear. Future studies are needed to elucidate the specific phytochemicals and molecular pathways involved, alongside in vivo and clinical investigations to fully validate these therapeutic potentials.

Keywords: *Persea americana*, Anti-inflammatory, Antioxidant, Antihypertensive, In-vitro assay, Cardiovascular health and Ethnopharmacology.

I. Introduction

Herbal medicine has played a significant role in global healthcare, with approximately 80% of the world's population relying on plant-based remedies for primary healthcare needs (World Health Organization, 2019). The increasing interest in medicinal plants stems from their diverse bioactive compounds, which offer therapeutic potential for various diseases (Salmerón-Manzano *et al.*, 2020, Dar *et al.*, 2023). Among these, *Persea americana* (PA), commonly known as avocado, has gained attention due to its rich phytochemical profile and reported health benefits (Bhuyan *et al.*, 2019).

Persea americana is widely cultivated, with Mexico accounting for nearly 30% of global production (Tremocoldi *et al.*, 2018, Olas, 2024). Traditionally, different parts of the plant, including leaves, fruit, and seeds have been used in ethnomedicine for treating ailments such as parasitic infections, gastrointestinal disorders, and cardiovascular diseases (Maity *et al.*, 2023, Gavidia-Valencia *et al.*, 2024). Phytochemical analyses have identified key bioactive compounds, including flavonoids, phenolics, and terpenoids, which contribute to its antioxidant, anti-inflammatory, and antihypertensive properties (da Silva *et al.*, 2022).

The therapeutic relevance of PA has been further supported by studies evaluating its pharmacological effects in oxidative stress-related conditions. Oxidative stress, characterized by an imbalance between free radicals and antioxidants, has been implicated in numerous pathological conditions such as diabetes, cancer, and neurodegenerative diseases (Juan *et al.*, 2021). Antioxidants play a crucial role in mitigating oxidative damage by neutralizing reactive oxygen species (Sharifi-Rad *et al.*, 2020, Jomova *et al.*, 2023). Additionally, chronic inflammation has been linked to autoimmune disorders and cardiovascular complications (Furman *et al.*, 2019). Given the interplay between oxidative stress and inflammation in disease progression, compounds with dual antioxidant and anti-inflammatory properties may serve as potential therapeutic agents for hypertension and related disorders (Sorriento *et al.*, 2018).

Despite promising findings, the specific mechanisms underlying PA's bioactivity remain insufficiently explored. While previous studies have identified its phytochemicals and reported positive effects *in vitro* assays, limited research has investigated whether these effects translate into significant physiological benefits. The interactions between PA's bioactive compounds and molecular pathways involved in oxidative stress modulation, inflammation suppression, and hypertension regulation warrant further clarification (Gavidia-Valencia *et al.*, 2024). Moreover, understanding whether PA's therapeutic potential arises from a single dominant compound or a synergistic interaction among multiple phytochemicals is essential for optimizing its pharmacological applications.

This study aims to evaluate the *in vitro* antioxidant, anti-inflammatory, and antihypertensive activities of PA leaf extracts. Specifically, assays such as DPPH, nitric oxide, ABTS radical scavenging, metal chelation, reducing power, hydroxyl radical inhibition, 15-lipoxygenase enzyme inhibition, and angiotensin I-converting enzyme (ACE) suppression were conducted. Additionally, given the absence of detailed mechanistic insights, future research directions should focus on elucidating the biochemical pathways underlying PA's therapeutic effects. The findings from this study will provide a foundation for further *in vivo* investigations to validate PA's therapeutic potential.

II. Methodology

Plant Extraction

The extraction of *Persea americana* leaves was performed following the protocol outlined by Abubakar and Haque (2020) with modifications to optimize bioactive compound recovery. Fresh leaves were chopped, air-dried, and blended into a fine powder. The powdered material was initially defatted using n-hexane for 48 hours to remove lipid-soluble components. The defatted residue was subsequently dried and macerated in methanol for three days. The resulting mixture was filtered using a cotton sieve, and the filtrate was concentrated under reduced pressure using a rotary evaporator.

To further separate bioactive compounds based on polarity, liquid-liquid partitioning was conducted using a separating funnel, employing solvents of increasing polarity: n-hexane, chloroform, ethyl acetate, n-butanol, and aqueous-methanol. Each fraction was evaporated and stored at 4°C until further analysis. Extraction efficiency and yield percentages were recorded for each fraction to ensure reproducibility.

In vitro Antioxidant Assays

DPPH Free Radical Scavenging Assay

The antioxidant potential of the extracts was assessed using the 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) assay, following the method described by Turkoglu *et al.* (2007). A methanolic DPPH solution (0.2 mM) was

prepared, and 1 mL was mixed with 1 mL of varying extract concentrations (0.0156–0.250 mg/mL). The mixture was incubated at 25°C for 30 minutes, after which absorbance was measured at 516 nm using a spectrophotometer. The percentage inhibition (PI) was calculated as:

$$PI = [(A_{\text{control}} - A_{\text{extract}}) / A_{\text{control}}] \times 100$$

where A_{control} represents the absorbance of the control and A_{extract} represents the absorbance of the test sample. The IC₅₀ value was extrapolated from a standard calibration curve.

Nitric Oxide (NO) Scavenging Assay

Nitric oxide scavenging capacity was evaluated using the method reported by Garrat (1964). Samples were prepared in a reaction mixture containing sodium nitroprusside and incubated at room temperature for 60 minutes. The absorbance was recorded at 540 nm, and percentage inhibition was determined as follows:

$$PI = [(A_c - A_e) / A_c] \times 100$$

Where A_c is the control reading and A_e is the extract reading. The IC₅₀ was calculated from a standard curve.

Metal Chelating Ability Assay

The chelation ability of the extracts was determined using the method by Dinis *et al.* (1994). Samples were reacted with ferrozine and Fe²⁺ ions, and absorbance was measured at 562 nm using a BIO-RAD Model 680 microplate reader. The IC₅₀ value was obtained from the calibration curve.

Reducing Power Assay

The ability of PA extracts to reduce free radicals was assessed following the protocol by Oyaizu (1986). Extracts at concentrations ranging from 0.0156 to 0.250 mg/mL were incubated with potassium ferricyanide (1%), followed by trichloroacetic acid precipitation. The absorbance was measured at 700 nm using a microplate reader.

ABTS Radical Scavenging Assay

The antioxidant activity of the extracts against 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radicals was determined using the method by Re *et al.* (1999). Extracts were incubated with ABTS solution, and absorbance was recorded at 734 nm using a BIO-RAD microplate reader, with gallic acid as the standard reference.

Hydroxyl Radical Inhibition Assay

Hydroxyl radical scavenging ability was evaluated following the method of Oboh *et al.* (2007). Extracts were incubated with hydroxyl-generating reagents, and absorbance was recorded at 532 nm. The IC₅₀ was calculated using a standard curve with gallic acid as the reference compound.

Anti-inflammatory Assay (*In vitro*)

The anti-inflammatory potential of PA extracts was assessed using 15-lipoxygenase enzyme inhibition, following Adebayo *et al.* (2015). Samples were incubated with linoleic acid substrate, and absorbance was recorded at 234 nm. The IC₅₀ was determined from the inhibition curve.

Angiotensin I-Converting Enzyme (ACE) Inhibition Assay

ACE inhibition activity was examined using modified protocols from Oboh *et al.* (2012) and Lin & Li (2012). PA extracts were reacted with **hippuryl-histidyl-leucine substrate**, and absorbance was recorded at **228 nm** using a **UNICO UV-2102 spectrophotometer**. The IC₅₀ was calculated based on inhibition curves.

III. Results

In vitro Antioxidant Activity of *Persea americana* Leaf Extracts Compared to Gallic Acid

The *in vitro* antioxidant activity of *Persea americana* leaf extracts is presented in Table 1. Among the tested fractions, the n-butanol fraction (PAB) exhibited the strongest ABTS radical scavenging ability, with an IC₅₀ of 12.93±1.10, which was significantly different ($p < 0.05$) from the methanol extract (PAM) (104.03±11.93) and chloroform fraction (PAC) (130.32±23.69). Notably, all fractions displayed a superior ABTS scavenging ability compared to gallic acid (IC₅₀ = 946.64±29.93), a well-known standard antioxidant.

Additionally, the DPPH radical scavenging assay revealed that PAB exhibited the highest scavenging potential (IC₅₀ = 24.67±5.13), outperforming the standard antioxidant (IC₅₀ = 113.57±6.64) and showing significant differences from other fractions. The aqueous-methanol fraction (PAA) demonstrated the most potent hydroxyl radical scavenging ability with an IC₅₀ of 25.40±7.88.

Moreover, different fractions excelled in specific antioxidative mechanisms: the chloroform fraction (PAC) exhibited the strongest metal chelating ability ($IC_{50} = 67.24 \pm 14.18$), while the n-hexane fraction (PAN) showed the most effective nitric oxide inhibition ($IC_{50} = 64.26 \pm 18.77$). These results indicate the diverse antioxidant capabilities of *P. americana* fractions, highlighting their potential application in oxidative stress-related conditions.

Table 1: *In vitro* Antioxidant Activity of *Persea americana* Leaf Extracts Compared to Gallic Acid

EXTRACTS /ASSAYS	ABTS	DPPH	HYDROXYL	METAL CHELATING	NITRIC OXIDE
A: PAM	104.03±11.93	110.63±0.49	108.76±14.39	67.24±14.18	105.64±8.32
B: PAN	59.66±13.08	97.65±0.17	420.77±43.97 ^a	148.11±33.38	64.26±18.77
C: PAC	130.32±23.69 ^b	309.79±2.67ab	33.61±4.70 ^{ab}	269.57±27.55 ^{ab}	296.88±6.44 ^b
D: PAE	70.61±13.79	95.02±0.12 ^c	119.37±17.98 ^{bc}	121.13±6.02 ^c	114.14±12.98
E: PAB	12.93±1.10 ^{ac}	24.67±5.13 ^{abcd}	258.73±7.27 ^{abcd}	159.20±5.67 ^{ac}	673.22±12.22 ^{abcd}
F: PAA	165.92±14.99 ^{abde}	212.11±4.51 ^{abde}	25.40±7.88 ^{abde}	137.89±4.64 ^c	332.01±33.72 ^{abde}
G: GA	946.64±29.93 ^c	113.57±6.64 ^{cef}	58.71±1.94 ^{bce}	184.09±12.73 ^a	220.29±12.38 ^{abde}

Group A: PA methanol extract (PAM), Group B: PA n-hexane fraction (PAN), Group C: PA chloroform fraction (PAC), Group D: PA ethyl acetate fraction (PAE), Group E: PA n-butanol fraction (PAB), Group F: PA aqueous-methanol fraction (PAA), Group G: Gallic acid (GA). Values are presented as IC_{50} (mean±SD). Means with superscript a-g show significance ($p < 0.05$) when compared with groups A-G, respectively.

***In vitro* Reducing Power of *Persea americana* Extracts in Comparison with Gallic Acid**

The *in vitro* reducing power of *Persea americana* leaf extracts and fractions, in comparison with gallic acid, is presented in Table 2. All tested fractions exhibited a dose-dependent increase in reducing power, indicating their ability to donate electrons and neutralize oxidative intermediates. However, the n-butanol fraction (PAB) reached its peak activity at a concentration of 125 μ g/mL, showing no further increase beyond this point.

Despite some variations in response, the reducing power of most fractions was comparable to that of gallic acid, a well-established standard antioxidant. These findings suggest that *P. americana* extracts possess strong electron-donating properties, reinforcing their potential therapeutic applications in oxidative stress-related conditions.

Table 2: *In vitro* Reducing Power of *Persea americana* Extracts in Comparison with Gallic Acid

CONC.	A: PAM	B: PAN	C: PAC	D: PAE	E: PAB	F: PAA	G: GA
15.63	0.029±0.02	0.29±0.03	0.28±0.01	0.38±0.03	0.42±0.01	0.330±0.01	0.460±0.05
31.25	0.32±0.02	0.28±0.01	0.297±0.03	0.46±0.04	0.65±0.10	0.396±0.01	0.504±0.08
62.50	0.36±0.03	0.32±0.01	0.41±0.73	0.64±0.07	0.74±0.01	0.480±0.02	0.746±0.04
125	0.52±0.04	0.45±0.02	0.51±0.02	0.96±0.02	0.95±0.01	0.640±0.01	1.048±0.02 ^b
250	0.54±0.26	0.70±0.03	0.67±0.00	0.96±0.01	0.93±0.01	0.850±0.01	1.325±0.06 ^{abc}

Group A: PA methanol extract (PAM), Group B: PA n-hexane fraction (PAN), Group C: PA chloroform fraction (PAC), Group D: PA ethyl acetate fraction (PAE), Group E: PA n-butanol fraction (PAB), Group F: PA aqueous-methanol fraction (PAA), Group G: Gallic acid (GA). Values are presented as IC_{50} (mean±SD). Means with superscript a-g show significance ($p < 0.05$) when compared with groups A-G, respectively.

***In vitro* Anti-inflammatory Effect of *Persea americana* Extracts and Fractions**

The *in vitro* anti-inflammatory activity of *Persea americana* extracts and fractions is summarized in Table 3. The results indicate that the n-butanol fraction (PAB) exhibited the highest inhibition of 15-lipoxygenase, with an IC_{50} value of 75.36 ± 4.03 , significantly outperforming the standard anti-inflammatory drug, indomethacin ($IC_{50} = 115.09 \pm 1.62$). However, its potency remained lower than quercetin ($IC_{50} = 59.33 \pm 5.4$), a well-known flavonoid with strong anti-inflammatory effects.

These findings highlight the potential of *P. americana* extracts as natural anti-inflammatory agents, with the PAB fraction demonstrating the strongest inhibitory activity. The results suggest that bioactive compounds within *P. americana* may effectively modulate inflammatory pathways, warranting further investigation into their mechanisms of action and potential therapeutic applications.

Table 3: *In vitro* Anti-inflammatory Activity of *Persea americana* Extracts Compared to Standard Anti-inflammatory Agents

Test samples	IC ₅₀
A: PAM	191.53 ±15.2
B: PAN	100.24±7.63 ^a
C: PAC	106.42±0.81 ^a
D: PAE	100.86±2.77 ^a
E: PAB	75.36±4.03 ^a
F: PAA	96.65±8.12 ^a
G: Quercetin	59.33±5.4 ^{abcdf}
H: Indomethacin	115.09±1.62 ^{acg}

Group A: PA methanol extract (PAM), Group B: PA n-hexane fraction (PAN), Group C: PA chloroform fraction (PAC), Group D: PA ethyl acetate fraction (PAE), Group E: PA n-butanol fraction (PAB), Group F: PA aqueous-methanol fraction (PAA), Group G: Quercetin, Group H: Indomethacin. Values are presented as IC₅₀ (mean±SD). Means with superscript a-g show significance (p<0.05) when compared with groups A-G, respectively.

The *In vitro* Antihypertensive Activity of *Persea americana* Extracts and Fractions

The *in vitro* antihypertensive activity of *Persea americana* extracts and fractions is illustrated in Figure 1. The study assessed their angiotensin I-converting enzyme (ACE) inhibitory potential, measured as percentage inhibition. The results indicate that the IC₅₀ value of the PA extract was significantly lower (p<0.05) compared to the standard antihypertensive drug, captopril, suggesting moderate ACE inhibition.

While the PA extract demonstrated notable inhibitory activity, its potency was lower than captopril, which is a well-established ACE inhibitor used in hypertension treatment. These findings highlight the potential of *P. americana* as a natural antihypertensive agent, though further investigation is needed to evaluate its mechanism of action, bioavailability, and effectiveness *in vivo*.

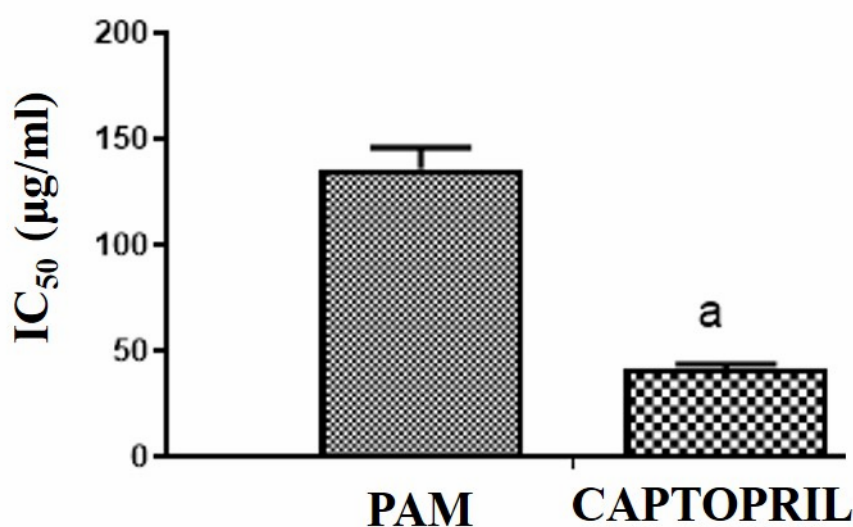


Figure 1: *In vitro* Angiotensin I-Converting Enzyme (ACE) Inhibitory Activity of *Persea americana* Extract Compared to Captopril

Group A: PA crude methanol leaf extract (PAM). Group B: Captopril (standard ACE inhibitor). Statistical significance was determined at $\alpha = 0.05$, indicating that the antihypertensive activity of captopril was significantly higher (p < 0.05) compared to PAM.

IV. Discussion

The study investigated the antioxidant, anti-inflammatory, reducing power, and antihypertensive effects of *Persea americana* leaf extracts and their fractions, revealing significant bioactivity across multiple assays.

These findings align with previous research highlighting the therapeutic potential of plant-derived compounds in mitigating oxidative stress, inflammation, and hypertension (Muhammad *et al.*, 2024, Pereira *et al.*, 2025).

The antioxidant activity of *Persea americana* leaf extracts demonstrated in this study aligns with previous research highlighting the plant's strong radical scavenging potential. The PAB fraction exhibited the highest ABTS scavenging ability ($IC_{50} = 12.93 \pm 1.10$), significantly outperforming gallic acid ($IC_{50} = 946.64 \pm 29.93$). This supports findings by Rahman *et al.* (2018), who reported potent DPPH radical scavenging activity in *Persea americana* leaf extracts ($IC_{50} = 72.61$ mg/L). Similarly, Kupnik *et al.* (2023) found that avocado seed extracts exhibited notable antioxidant potential, with a DPPH scavenging efficiency of 67.49%. These results suggest that avocado-derived compounds contain bioactive molecules capable of neutralizing free radicals.

Furthermore, the hydroxyl radical scavenging ability of PAA ($IC_{50} = 25.40 \pm 7.88$) aligns with findings from Hussen and Endalew (2023), who reported comparable antioxidant activity in *Vernonia amygdalina* leaf extracts (DPPH and ABTS IC_{50} values ranging from 94.83 to 334.3 μ g/mL). These similarities suggest that *P. americana* may contain phenolic compounds that contribute to its antioxidant effects.

The metal chelating activity of PAC ($IC_{50} = 67.24 \pm 14.18$) and nitric oxide inhibition by PAN ($IC_{50} = 64.26 \pm 18.77$) further highlight the diverse antioxidative mechanisms exhibited by different fractions. Christodouleas *et al.* (2015) discussed the importance of solvent selection in antioxidant profiling and emphasized the significance of ABTS and DPPH assays in determining radical scavenging efficiency, reinforcing the results obtained in this study.

These results confirm that *Persea americana* leaf extracts exhibit strong antioxidant activity, comparable to findings in previous literature. Future research should focus on identifying specific bioactive compounds responsible for these effects, optimizing extraction methods, and evaluating their therapeutic potential in oxidative stress-related diseases.

Furthermore, the reducing power of *Persea americana* leaf extracts observed in this study highlights their electron-donating ability, which plays a crucial role in neutralizing oxidative intermediates. The dose-dependent increase in reducing power across all fractions suggests that these extracts can effectively donate electrons to stabilize free radicals, a key mechanism in antioxidant defense. However, the n-butanol fraction (PAB) peaked at 125 μ g/mL, indicating a saturation point beyond which its reducing power does not further improve (Yamassaki *et al.*, 2017).

These findings align with previous studies on polyphenolic antioxidants, such as those reported by Yamassaki *et al.*, (2017) who demonstrated that *Persea americana* leaf extracts contain flavonol glycosides and flavan-3-ols, which contribute to their reducing power and radical scavenging activity (Yamassaki *et al.*, 2017). Similarly, Antasionasti *et al.* (2017) found that avocado peel extracts exhibited strong reducing power, comparable to ascorbic acid, reinforcing the electron-donating potential of avocado-derived compounds (Antasionasti *et al.*, 2017).

The reducing power of most *P. americana* fractions was comparable to gallic acid, a well-established standard antioxidant. This supports findings by Chen *et al.*, (2020) who investigated the structure-antioxidant activity relationship of phenolic acids and found that methoxy and hydroxyl groups enhance electron donation, contributing to higher reducing power (Chen *et al.*, 2020). Additionally, Walpen *et al.* (2016) quantified phenolic antioxidant moieties in dissolved organic matter, confirming that electron-donating phenolic compounds play a vital role in oxidation-reduction reactions (Walpen *et al.*, 2016).

Our results reveals that *P. americana* extracts possess strong electron-donating properties, reinforcing their potential therapeutic applications in oxidative stress-related conditions. Future studies should explore synergistic effects with other antioxidants, optimize extraction methods, and evaluate their bioavailability in biological systems (Walpen *et al.*, 2016).

The anti-inflammatory activity of *Persea americana* leaf extracts observed in this study aligns with previous research demonstrating the plant's ability to modulate inflammatory pathways. The n-butanol fraction (PAB) exhibited the highest inhibition of 15-lipoxygenase ($IC_{50} = 75.36 \pm 4.03$), significantly outperforming indomethacin ($IC_{50} = 115.09 \pm 1.62$), a widely used anti-inflammatory drug. However, its potency remained lower than quercetin ($IC_{50} = 59.33 \pm 5.4$), a flavonoid known for its strong anti-inflammatory effects (Kristanti *et al.*, 2017).

These findings are consistent with studies highlighting the anti-inflammatory potential of avocado-derived compounds. Kristanti *et al.* (2017) reported that methanolic extracts of *Persea americana* seeds significantly reduced inflammation in carrageenan-induced paw edema models, suggesting the presence of bioactive compounds capable of modulating inflammatory responses. Similarly, Sakshi *et al.* (2023) reviewed the pharmacological properties of *P. americana* and emphasized its role in reducing inflammation through polyphenolic compounds, which may contribute to lipoxygenase inhibition.

The mechanism of 15-lipoxygenase inhibition by *P. americana* extracts warrants further investigation. Meng *et al.* (2018) explored the molecular mechanisms of 15-lipoxygenase inhibition, demonstrating that

allosteric inhibitors can prevent substrate oxidation and reduce inflammatory mediator production. Given the strong lipoxygenase inhibitory activity observed in the PAB fraction, future studies should focus on identifying specific bioactive compounds responsible for this effect and evaluating their potential for therapeutic applications.

Additionally, Cakir-Aktas *et al.* (2023) reported that 12/15-lipoxygenase inhibition attenuates neuroinflammation by suppressing inflammasome activation, reinforcing the importance of lipoxygenase inhibitors in managing inflammatory conditions. The findings from this study suggest that *P. americana* extracts may serve as natural alternatives to synthetic anti-inflammatory drugs, particularly in conditions where lipoxygenase-mediated inflammation plays a key role.

The antihypertensive activity of *Persea americana* leaf extracts observed in this study suggests their potential role in modulating blood pressure through ACE inhibition. The results indicate that the IC₅₀ value of the PA extract was significantly lower ($p < 0.05$) compared to captopril, a widely used ACE inhibitor, though its potency remained lower than captopril. This aligns with previous studies demonstrating the ACE inhibitory potential of plant-derived bioactive compounds (Mutasa *et al.*, 2025).

Mutasa *et al.* (2025) reviewed the antihypertensive properties of *Persea americana*, *Myrothamnus flabellifolius*, and *Xeroderris stuhlmannii*, emphasizing their ability to regulate blood pressure through ACE inhibition and antioxidant mechanisms. Similarly, Djomeni Dzeufiet *et al.* (2014) investigated the antihypertensive effects of an aqueous extract combining *P. americana* leaves with *Cymbopogon citratus* and *Citrus medica*, demonstrating significant blood pressure reduction in ethanol- and sucrose-induced hypertensive rats. These findings reinforce the potential of *P. americana* as a natural antihypertensive agent, though further studies are needed to confirm its efficacy in human models.

Additionally, Gbadamosi and Kalejaye (2017) compared the antioxidant activity and phytochemical composition of *P. americana* and *Xylopia aethiopica*, highlighting their polyphenol content and radical scavenging ability. Since oxidative stress plays a key role in hypertension, the antioxidant properties of *P. americana* may contribute to its antihypertensive effects.

V. Conclusion

This study establishes the antioxidant, anti-inflammatory, and antihypertensive potential of *Persea americana* leaf extracts, emphasizing their therapeutic relevance in disease prevention and management. The PAB fraction consistently demonstrated superior bioactivity, particularly in radical scavenging and lipoxygenase inhibition, positioning *P. americana* as a valuable source of functional phytochemicals. While its ACE inhibitory properties suggest potential antihypertensive applications, further research must clarify its mechanisms, bioavailability, and *in vivo* efficacy.

Future studies should focus on identifying specific bioactive molecules, optimizing extraction techniques, and evaluating synergistic effects with conventional drugs. Expanding its clinical applications could enhance its therapeutic potential in oxidative stress-related diseases, inflammation, and hypertension management, providing a promising natural alternative to pharmaceutical interventions.

References

- [1]. Abubakar, A. R., & Haque, M. (2020). Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. *Journal of Pharmacy and Bioallied Sciences*, 12(1), 1–10. https://doi.org/10.4103/jpbs.JPBS_175_19
- [2]. Adebayo, S. A., Dzoyem, J. P., Shai, L. J., & Eloff, J. N. (2015). The anti-inflammatory and antioxidant activity of 25 plant species used traditionally to treat pain in southern Africa. *BMC Complementary Medicine and Therapies*, 15, Article 159. <https://doi.org/10.1186/s12906-015-0669-5>
- [3]. Antasionasti, I., Riyanto, S., & Rohman, A. (2017). Antioxidant activities and phenolic contents of avocado (*Persea americana* Mill.) peel *in vitro*. *Research Journal of Medicinal Plants*, 11(2), 55–61. <https://doi.org/10.3923/rjmp.2017.55.61>
- [4]. Bhuyan, D. J., Alsherbiny, M. A., Perera, S., Low, M., Basu, A., & Barooah, P. (2019). Avocado (*Persea americana*) fruit and by-products as potential sources of bioactive compounds. *Journal of Food Science and Technology*, 56(5), 2217–2227. <https://doi.org/10.1007/s13197-019-03685-5>
- [5]. Cakir-Aktas, C., Bodur, E., Yemisci, M., van Leyen, K., & Karatas, H. (2023). 12/15-lipoxygenase inhibition attenuates neuroinflammation by suppressing inflammasomes. *Frontiers in Cellular Neuroscience*, 17, Article 1277268. <https://doi.org/10.3389/fncel.2023.1277268>
- [6]. Chen, J., Yang, J., Ma, L., Li, J., Shahzad, N., & Kim, C. K. (2020). Structure-antioxidant activity relationship of methoxy, phenolic hydroxyl, and carboxylic acid groups of phenolic acids. *Scientific Reports*, 10, Article 59451. <https://doi.org/10.1038/s41598-020-59451-z>
- [7]. Christodouleas, D. C., Fotakis, C., Nikokavoura, A., Papadopoulos, K., & Calokerinos, A. C. (2015). Modified DPPH and ABTS assays to assess the antioxidant profile of untreated oils. *Food Analytical Methods*, 8, 1294–1302. DOI: 10.1007/s12161-014-0005-6
- [8]. Dar, R. A., Shah Nawaz, M., Ahanger, M. A., & Majid, I. U. (2023). Exploring the Diverse Bioactive Compounds from Medicinal Plants: A Review. *The Journal of Phytopharmacology*, 12(3), 189–195. <https://doi.org/10.31254/phyto.2023.12307>
- [9]. da Silva GG, Pimenta LPS, Melo JOF, Mendonça HOP, Augusti R, Takahashi JA. Phytochemicals of Avocado Residues as Potential Acetylcholinesterase Inhibitors, Antioxidants, and Neuroprotective Agents. *Molecules*. 2022 Mar 15;27(6):1892. doi: 10.3390/molecules27061892. PMID: 35335256; PMCID: PMC8953789.

- [10]. Dinis, T. C. P., Madeira, V. M. C., & Almeida, L. M. (1994). Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Archives of Biochemistry and Biophysics*, 315(1), 161–169. <https://doi.org/10.1006/abbi.1994.1485>
- [11]. Djomeni Dzeufiet, P. D., Mogueo, A., Bilanda, D. C., Aboubakar, B. F. O., Tédong, L., Dimo, T., & Kamtchouing, P. (2014). Antihypertensive potential of the aqueous extract combining *Persea americana* leaves, *Cymbopogon citratus*, and *Citrus medica* in ethanol- and sucrose-induced hypertensive rats. *BMC Complementary Medicine and Therapies*, 14, Article 507. <https://doi.org/10.1186/1472-6882-14-507>.
- [12]. Furman D, Campisi J, Verdin E, Carrera-Bastos P, Targ S, Franceschi C, Ferrucci L, Gilroy DW, Fasano A, Miller GW, Miller AH, Mantovani A, Weyand CM, Barzilai N, Goronzy JJ, Rando TA, Effros RB, Lucia A, Kleinstreuer N, Slavich GM. Chronic inflammation in the etiology of disease across the life span. *Nat Med*. 2019 Dec;25(12):1822-1832. doi: 10.1038/s41591-019-0675-0. Epub 2019 Dec 5. PMID: 31806905; PMCID: PMC7147972.
- [13]. Garrat D.C. (1964). Quantitative Analysis of drugs Japan 3rd Edn; Chapman and Hall, Japan pp 456-458.
- [14]. Gavidia-Valencia, J. G., Venegas-Casanova, E. A., Gutiérrez-Ramos, M. E., Rengifo-Penadillos, R. A., Cajamarca-Porras, C. J., Aro-Díaz, R. J., Mostacero-León, J., & Albuquerque, R. D. G. (2024). Traditional use, phytoconstituents, and pharmacological effects of *Persea americana*: A recent review. *Journal of Applied Biology*, 5(4), 119–127. https://jabonline.in/admin/php/uploads/1256_pdf.pdf.
- [15]. Gbadamosi, I. T., & Kalejaye, A. O. (2017). Comparison of the antioxidant activity, phytochemical and nutritional contents of two antihypertensive ethnomedicinal plants. *Ife Journal of Science*, 19(1), 147–158. DOI:10.4314/ijfs.v19i1.15.
- [16]. Hussien, E. M., & Endalew, S. A. (2023). In vitro antioxidant and free-radical scavenging activities of polar leaf extracts of *Vernonia amygdalina*. *BMC Complementary Medicine and Therapies*, 23, Article 146. DOI: 10.1186/s12906-023-03923-y.
- [17]. Jomova, K., Raptova, R., Alomar, S. Y., Alwasel, S. H., Nepovimova, E., Kuca, K., & Valko, M. (2023). Reactive oxygen species, toxicity, oxidative stress, and antioxidants: Chronic diseases and aging. *Archives of Toxicology*, 97, 2499–2574. <https://doi.org/10.1007/s00204-023-03562-9>.
- [18]. Juan CA, Pérez de la Lastra JM, Plou FJ, Pérez-Lebeña E. The Chemistry of Reactive Oxygen Species (ROS) Revisited: Outlining Their Role in Biological Macromolecules (DNA, Lipids and Proteins) and Induced Pathologies. *Int J Mol Sci*. 2021 Apr 28;22(9):4642. doi: 10.3390/ijms22094642. PMID: 33924958; PMCID: PMC8125527.
- [19]. Kristanti, C. D., Simanjuntak, F. P. J., Dewi, N. K. P. A., Tianri, S. V., and Hendra, P. (2017). Anti-inflammatory and analgesic activities of avocado seed (*Persea americana* Mill.). *Journal of Pharmaceutical Sciences and Community*, 14(2), 104-111.
- [20]. Kupnik, S., Primožič, M., Kokol, V., Knez, Ž., & Leitgeb, M. (2023). Enzymatic, antioxidant, and antimicrobial activities of bioactive compounds from avocado (*Persea americana* L.) seeds. *Plants*, 12(5), Article 1201. DOI: 10.3390/plants12051201.
- [21]. Maity, A., Mondal, B. M., Pan, D., Mukherjee, P. A., Mondal, M., & Khalua, R. K. (2023). Biochemistry and nutritional health benefits of avocado (*Persea americana*): An overview. *African Journal of Biological Sciences*, 5(4), 119–127. <https://doi.org/10.33472/AJBS.5.4.2023.119-127>.
- [22]. Meng, H., Dai, Z., Zhang, W., Liu, Y., & Lai, L. (2018). Molecular mechanism of 15-lipoxygenase allosteric activation and inhibition. *Physical Chemistry Chemical Physics*, 21(7), 8586-8598. <https://doi.org/10.1039/C7CP08586A>.
- [23]. Muhammad, A., Chukwuma, C. I., Erukainure, O. L., & Islam, M. S. (2024). Therapeutic potential of natural products in oxidative and metabolic diseases. *Front. Pharmacol.*, 15, 1375788. <https://doi.org/10.3389/fphar.2024.1375788>.
- [24]. Mutasa, I., Kademeteme, E., Pote, W., Gundidza, M., Nyabadza, B. L., et al. (2025). A mini review on the antihypertensive properties of *Persea americana*, *Myrothamnus flabellifolius*, and *Xeroderris stuhlmannii*. *American Journal of Biomedical Science & Research*, 26(1). <https://doi.org/10.34297/AJBSR.2025.26.00341>.
- [25]. Oboh, G., Puntel, R.L., Rocha, J.B.T.I., (2007) Hot pepper (*Capsicum annum*, *Tepin* and *Caspicum chinese*, *Habanero*) prevents Fe induced lipid peroxidation in brain- *in vitro*, Food chemistry, Vol 102, (1), 178-185.
- [26]. Oboh, G., Ademiluyi, A.O., Akinyemi, A.J., Henle T., Saliu J.A. and Schwarzenbolz, U. (2012). Inhibitor effect of polyphenol-rich extracts of jute leaf (*Corchorus olitorius*) on key enzyme linked to type 2 diabetes (α-amylase and α-glycosidase) and hypertension (Angiotensin I Converting) *in vitro*. *J. Funct Foods* 4: 450-458.
- [27]. Olas B. The Pulp, Peel, Seed, and Food Products of *Persea americana* as Sources of Bioactive Phytochemicals with Cardioprotective Properties: A Review. *International Journal of Molecular Sciences*. 2024; 25(24):13622. <https://doi.org/10.3390/ijms252413622>.
- [28]. Oyaizu, M. (1986). Studies on products of browning reaction: Antioxidative activities of products of browning reaction prepared from glucosamine. *The Japanese Journal of Nutrition*, 44(6), 307–315. <https://doi.org/10.5264/eiyogakuzashi.44.307>.
- [29]. Pereira, A. G., Echave, J., Jorge, A. O. S., Nogueira-Marques, R., Nur Yuksek, E., Barciela, P., Perez-Vazquez, A., Chamorro, F., P. P. Oliveira, M. B., Carpena, M., & Prieto, M. A. (2025). Therapeutic and Preventive Potential of Plant-Derived Antioxidant Nutraceuticals. *Foods*, 14(10), 1749. <https://doi.org/10.3390/foods14101749>.
- [30]. Rahman, N., Dewi, N. U., & Bohari. (2018). Phytochemical and antioxidant activity of avocado leaf extract (*Persea americana* Mill.). *Asian Journal of Scientific Research*, 11(3), 357-363. DOI: 10.3923/ajsr.2018.357.363.
- [31]. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, 26(9-10), 1231–1237. [https://doi.org/10.1016/S0891-5849\(98\)00315-3](https://doi.org/10.1016/S0891-5849(98)00315-3).
- [32]. Sakshi P, Raibarinder S, Harsh P, Ramica S. Potential Role of Avocado (*Persea americana*) . *Glob J Pharmaceu Sci*. 2023; 10(5): 555797. DOI: 10.19080/GJPPS.2023.10.555797.
- [33]. Salmerón-Manzano E, Garrido-Cardenas JA, Manzano-Agugliaro F. Worldwide Research Trends on Medicinal Plants. *Int J Environ Res Public Health*. 2020 May 12;17(10):3376. doi: 10.3390/ijerph17103376. PMID: 32408690; PMCID: PMC7277765.
- [34]. Sharifi-Rad M, Anil Kumar NV, Zucca P, Varoni EM, Dini L, Panzarini E, Rajkovic J, Tsouh Fokou PV, Azzini E, Peluso I, Prakash Mishra A, Nigam M, El Rayess Y, Beyrouthy ME, Polito L, Iriti M, Martins N, Martorell M, Docoa AO, Setzer WN, Calina D, Cho WC, Sharifi-Rad J. Lifestyle, Oxidative Stress, and Antioxidants: Back and Forth in the Pathophysiology of Chronic Diseases. *Front Physiol*. 2020 Jul 2;11:694. doi: 10.3389/fphys.2020.00694. PMID: 32714204; PMCID: PMC7347016.
- [35]. Sorriento D, De Luca N, Trimarco B, Iaccarino G. The Antioxidant Therapy: New Insights in the Treatment of Hypertension. *Front Physiol*. 2018 Mar 21;9:258. doi: 10.3389/fphys.2018.00258. PMID: 29618986; PMCID: PMC5871811.
- [36]. Tremocoldi M.A., Rosalen P.L., Franchin M., Massarioli A.P., Denny C., Daiuto É.R., Paschoal J.A.R., Melo P.S., Alencar S.M. Exploration of avocado by-products as natural sources of bioactive compounds. *PLoS One*. 2018 Feb 14;13(2):e0192577. doi: 10.1371/journal.pone.0192577. PMID: 29444125; PMCID: PMC5812635.
- [37]. Turkoglu, A., Duru, M. E., Mercan, N., Kivrak, I., & Gezer, K. (2007). Antioxidant and antimicrobial activities of *Laetiporus sulphureus* (Bull.) Murill. *Food Chemistry*, 101(1), 267–273. <https://doi.org/10.1016/j.foodchem.2006.01.025>.

- [38]. Walpen, N., Schroth, M. H., & Sander, M. (2016). Quantification of phenolic antioxidant moieties in dissolved organic matter by flow-injection analysis with electrochemical detection. *Environmental Science & Technology*, 50(11), 5798-5807. <https://doi.org/10.1021/acs.est.6b01120>.
- [39]. World Health Organization. (2019). WHO global report on traditional and complementary medicine. *World Health Organization Publications*. Available at <https://www.who.int/publications/i/item/978924151536>. accessed on June 12, 2025.
- [40]. Yamassaki, F. T., Campestrini, L. H., Zawadzki-Baggio, S. F., & Maurer, J. B. B. (2017). Influence of drying process, thermal incubation, and storage conditions on preservation of polyphenolic compounds and antioxidant activity in *Persea americana* leaves. *International Journal of Food Properties*, 20(S2), S2280–S2293. <https://doi.org/10.1080/10942912.2017.1369105>.