THERAPEUTIC EFFICACY AND MECHANISMS OF ACTION OF PERSEAAMERICANA EXTRACTS ON HYPERTENSION AND CARDIO-RENAL OXIDATIVE STRESS INDUCED BY N^{Ω}-NITRO-L-ARGININE METHYL ESTER

BY

ADEJUMOBI OLUMUYIWA ABIOLA

D.V.M., MVSc. (IBADAN), M.B.A. (O.A.U), FCVSN

MATRIC NO: 66534

BEING A THESIS IN THE

DEPARTMENT OF VETERINARY MEDICINE

SUBMITTED TO THE FACULTY OF VETERINARY MEDICINE

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

OF THE

UNIVERSITY OF IBADAN, IBADAN, NIGERIA

AUGUST, 2021

CERTIFICATION

We confirm that Adejumobi Olumuyiwa Abiola carried out this work in the Department of Veterinary Medicine, University of Ibadan, under our supervision.

……

SUPERVISOR

Prof. Helen O. Nottidge

D.V.M., MVSc., PhD (IB), FCVSN

Department of Veterinary Medicine,

University of Ibadan. Ibadan.

……..

SUPERVISOR

Dr. T.O. Omobowale

D.V.M., MVSc., PhD (IB), FCVSN

Department of Veterinary Medicine,

University of Ibadan. Ibadan.

DEDICATION

This work is devoted to God, my Creator and Lord, who has given me the grace to complete this study. Also to my family for their sacrifice and perseverance in the course of this study.

ACKNOWLEDGEMENT

My sincere appreciation goes to the Almighty God, in whom all blessings flow, for His support, provision and kindness. I am grateful to my Supervisor mentor and mother, Prof. Helen Nottidge for her tutelage, words of encouragement and support. Mama is wonderful caring, kind and loving. Her contribution to the success of this word is invaluable. I also greatly appreciate my co-supervisor, Dr T.O Omobowale, who is my Head of Unit and Head of Department. He is my father, he took me from the cradle and set me on the part of career excellence. I cannot appreciate him enough for his invaluable contribution to this work. I pray that God will multiply you and all your's. It is my heartfelt prayers that God will give you all your due rewards for your hard works. I appreciate my late father, Rev'd S.A Adejumobi and Mum, Mrs C.A Adejumobi for their prayers. Mummy, I pray that God will give you good health to enjoy the fruits of your labour. My gratitude also goes to my wife who taught me to absolutely trust in God. I appreciate Testimony, TiOluwanimi, TiKristinimi and TiOluwalase, my children, for their love and patience. My gratitude goes to Prof. Adeolu Adedapo and Dr Oyagbemi Ademola for the opportunity to use the cardio - renal laboratory and all the support all the way. I appreciate Dr Jerry Afolabi and Blessing Ogunpolu for their support during this study.

I am grateful to Prof. C.A.O. Adeyefa, Prof. Omolade Oladele and Late Prof. A.A Owoade, Late Dr Ayoade, Dr O.T. Jeremiah, and Dr Oladosu for being wonderful and their genuine interest in my progress. I appreciate Drs Omotosho, Olusoji Abiola, S. Olaogun, and Seun Esan who are my colleagues and fellows in the department of Veterinary Medicine for their encouragement and support. I also appreciate Mrs Akoma, Mrs Ofarn, Mrs Dupe Aroyeun, Mr John Omoreghe and other members of staff for their encouragement. I am indebted to Professor Robert Murphy of Northwestern University, Chicago, USA, Prof. Yakubu Momoh of Texas Southern University, Houston, U.S.A., Prof Sakthivel Sadayapan of the University of Cincinnati, Dr T.O.M. Ashafa of the University of Free State, QwaQwa, South Africa for their immense financial, technical, laboratory facilities, reagents and training support in the course of this study. May God bless you all and spare your lives. I appreciate Profs. G.A. T. Ogundipe, R.O. Arowolo and M. Oyeyemi for their support and encouragement. I appreciate Prof J.O. Olopade for his encouragement and support. I am also grateful to my teachers, supervisors and entire staff of the Faculty.

I am indebted to TETfund Nigeria for the grant under Academic staff development fund programme, given me for my bench work in the United States of America. I also appreciate Late Oba Sansi and Rev'd and Mrs Olubi Johnson for the Oba sansi staff development grant for my trip to the South Africa for some aspects of this study.

I appreciate the Vice Chancellor and the management of the University for approving my leave and for giving me the opportunity to fulfil my dreams. May God continue to bless them. May the university grow from strength to strength.

On a final note, I am grateful to all that contributed in one way or the other to the success of this work that time and space did not permit me to mention.

ABSTRACT

Cardiovascular diseases are the leading causes of death worldwide with hypertension being the major risk factor. The adverse effects of synthetic medications have made the search for safer and effective herbal therapies imperative. *Persea americana*(PA) is a medicinal plant with diverse health benefits. There is dearth of information on its safety and efficacy in the management of hypertension.The safety and ameliorative effects of PA extractson N^{Ω}-Nitro-L-Arginine Methyl Ester (L-NAME) induced hypertension and cardiorenal oxidative stress were investigated.

In vitro anti-inflammatory and anti-hypertensive assays were carried out separately on methanol PA leaf (PALE), stem bark (PABE), and root (PARE) extracts (UIH22531). Standard *in vitro* protocols were adopted using gallic acid, indomethacin and captopril, respectively, as standard drugs. Half maximum Inhibition Concentrations (IC_{50}) were calculated using IC_{50} calibration curve. Male Wistar rats (7 groups, n=4/group) received distilled water (control) and variable doses of PA extracts (1000, 2000, 4000, 5000, 6000 and 7500 mg/kg) once to determine the Lethal Dose (LD_{50}). Antihypertensive effect of PA was evaluated using 120 male rats randomly allotted to 12 groups (n=10). These received distilled water, L-NAME (40 mg/kg), L-NAME+ PALE (100, 200, 400 mg/kg), L-NAME + PABE (100, 200, 400 mg/kg) and L-NAME + PARE (100, 200, 400 mg/kg) and L-NAME + lisinopril (0.28 mg/kg). Blood pressure (BP) measurement and electrocardiogram were performed. Immunoreactivity of NF-κB, marker of oxidative stress: Malondialdehyde (MDA) and antioxidants: Superoxide dismutase(SOD) and Reduced Glutathione (GSH) were evaluated in the cardiac and renal tissues. Data were analysed using descriptive statistics and ANOVA at $\alpha_{0.05}$.

Persea americana extracts demonstrated anti-inflammatory activities with IC_{50} of the PALE (100.86±2.77), PABE (101.54±9.01) and PARE (101.74±13.10) comparable to Indomethacin (115.09±1.62). The extracts had anti-hypertensive activities with IC_{50} of 135.99±10.06, 29.64±0.92, 48.61±6.56, respectively, for PALE, PABE and PARE comparable to captopril (IC_{50} = 41.86±2.10). LD_{50} (mg/kg) for PALE, PABE and PARE were 3162.30, 3463.90 and 5477.20, respectively. *Persea americana* extracts significantly reduced systolic BP (mmHg) from 224.33±11.89 to110±31.87 (PALE-200), 113.67±9.49 (PABE-200) and 111.33±17.84 (PARE-200). A significant increase in QT interval (ms) in hypertensive rats (106.00±1.00) compared to the control (45.33±26.03) was reduced to 62.00±11.53 (PALE-100), 67.67±11.66 (PABE-200) and 62.67±1.53 (PARE-100) better than lisinopril (86.00±10.64). Treatment of hypertensive rats with PA extracts caused significant reduction in NF-κB expression, cardiac MDA (µmol/mgprotein) from 0.87±0.25 to 0.44±0.08 (PALE-200), 0.55±0.14 (PABE-200) and 0.33±0.22 (PARE-200) as well as renal MDA from 1.11±0.04 to 0.41±0.14 (PALE-200), 0.60±0.19 (PABE-200) and 0.74±0.18 (PARE-200). Cardiac SOD (units/mgprotein) increased from 1.19±0.05 to24.03±5.74 (PALE-200), 15.12±6.07 (PABE-200) and 11.27±1.03 (PARE-200) and renal SOD from 5.27±4.13 to 7.59±0.99 (PALE-200), 16.19±0.85 (PABE-200) and 9.59±1.05 (PARE-200). Cardiac GSH (µmol/mgprotein) increased from 51.82±13.20 to 88.37±1.55 (PALE-200), 73.77±11.53 (PABE-200) and 68.86±7.99 (PARE-200) comparable to lisinopril (68.74±3.68).

Persea americana is safe and effective at 200 mg/kg, ameliorating hypertension induced cardio-renal damages through free radical scavenging and anti-inflammatory activities. Thus, the plant is recommendedfor the management of hypertension and cardio-renal dysfunction.

Keywords: Cardio-renal oxidative stress, *Persea americana*, Anti-inflammation, Anti-hypertensive

Word count: 483

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATION

L-NAME: N^{Ω}-Nitro-L-Arginine Methyl Ester

IC_{50}: Half Maximum Inhibitory Concentration

LD_{50}: Median Lethal Dose

GSH: Glutathione

GPx: Glutathione peroxidase

GST: Glutathione-S-transferase

SOD: Superoxide dismutase

CAT: Catalase

MDA: Malondialdehyde

PA: *Persea americana*

DPPH: 1, 1- Diphenyl–2 Picrylhydrazyl

ABTS: 2, 2' Azinobis 3-Ethylbenzothiazoline- 6-Sulfonic Acid

ACE: Angiotensin I converting enzyme

PCV: Packed Cell Volume

RBC: Red Blood Cell Count

WBC: White Blood Cell

Hb: Haemoglobin

MCV: Mean Corpuscular Volume

MCHC: Mean Corpuscular Haemoglobin Concentration

ALT: Alanine aminotransferase

AST: Aspartate Aminotransferase

PALE: *Persea americana* leaf extract

PABE: *Persea americana* bark extract

PARE: *Persea americana* root extract

TLC: Thin Layer chromatography

Prep- TLC: Preparative Thin Layer Chromatography

NMR: Nuclear Magnetic Resonance spectroscopy

FTIR: Fourier Transform Infrared Spectroscopy

CHAPTER ONE

1.0 INTRODUCTION

Cardiovascular diseases (CVDs) have been recognized as a major cause of death across the globe and they place enormous economic burden on patients, families and the national economy (Samb *et al*., 2010). This economic effect of CVDs is more pronounced in developing countries and this is aggravated by dysfunctional or moribund healthcare delivery system as well as lack of affordability. It is even made worse by the fact that a significant cost of the treatment is borne by the patients from their earnings (Samb *et al*., 2010).Over the last century, the incidence of CVDs have burgeoned from a disease of minor significance to that which is associated with high incidence of death and illness (Levenson *et al*., 2002). Decades ago, human death associated with CVDs was below 10% globally, however, in the early 21st century, 25% of all deaths was reported to be associated with CVDs in the developing countries. Also, 50 in 100 deaths in the developed world are attributed to CVDs. It was projected that by year 2020, about 25 million deaths worldwide would be caused by cardiovascular diseases (Levenson *et al*., 2002).

Hypertension (HBP) is a cardiovascular disease of increasing medical and public health importance reported in about one billion people of the world's population (Chobanian *et al*., 2003). It is a risk factor for myocardial infarction, stroke and chronic renal failure (WHO, 2002). Hypertension is a complex disease which is said to be triggered by many factors including genetic component and environment of an individual (Rafiq *et al*., 2010). Genes and the environment are major factors that coordinate vascular smooth mucle tone and excretion of sodium by the kidney. These invariably influence the structure and function of the heart and associated organs. (Giles *et al.*, 2005). Hypertension with its underlying mechanisms results in target organ damage resulting in dysfunction of the blood vessels and the kidney; manifesting as arteriosclerosis, neuropathy, hypertrophic cardiomyopathy, brain

ischaemia, heart and renal failure (Sandor & Thomas, 2012). Factors that are responsible for HBP include obesity, high salt consumption, sedentary, alcoholism, and inadequate consumption of fruits and vegetable. (Ogden *et al*., 2000). Unfortunately, hypertension can occur in individuals without their knowledge, and if left untreated can result in vascular, renal and brain damage. Untreated hypertension can even lead to myocardial infarction and vascular disease (Dreisbach, 2015). As a result, much has been invested on the awareness, management and treatment of hypertension, however, approximately 7.1 million annual death was still reported (Chobanian *et al*., 2003).

Various therapeutic drugs and drug combinations have been used to manage hypertension (Ogden *et al*., 2000). Their therapeutic effects wereaimed at reducing the cardiovascular or renal and other organs dysfunctions associated with the complications of hypertension, thereby preventing at least 1 death in every 11 hypertensive patient treated (Ogden *et al*., 2000) In some situations, patients require combination of drugs to effectively control HBP (Rimoy *et al*., 2008). The various classes of antihypertensive drugs used include: diuretics, antidiuresis inhibitors, angiotension converting enzyme inhibitors, calcium channel blockers and β-blockers (Rimoy *et al*., 2008).

Despite the high efficacy of these orthodox antihypertensives, various side and adverse effects have been documented (Loga-Zec *et al*., 2014). These include hypokalaemia, bronchoconstriction, peripheral vasoconstriction, hyperkalaemia, teratogenicity, renal failure, angioedema, hepatotoxicity, haemolytic anaemia, sedation, peptic ulcer, weight loss, etc. (Loga-Zec *et al*., 2014). Ethno-pharmacological plants documented to have anti-hypertensive properties have also been used in the management of hypertension. These include:*Hisbiscus Sabdarifa*, *Rhaptopetalum coriaceum oliv (Scytopetalaceae), Allium sativum (Garlic), Musanga cecropioides, Cassia occidentalis, Phyllanthus amarus (Eurphobiaceae), Lepidium Latifolium (Cruciferae),* linseed (*Linium usitatissumum* (linseed) amongst others (Etuk, 2006).Though these herbal plants have high efficacy in the management of hypertension, they have not been associated with the common complications associated with orthodox antihypertensive drugs (Frishman *et al*., 2009).They are also cheaper and widely available to a larger percentage of the world's population especially those in developing and underdeveloped countries.

Persea americana(PA) is a green leavy plant that is commonly called Avocado pear. It was discovered to be medicinal and potent against many health conditions (Owolabi *et al*., 2010; Ojewole, 2007). The avocado pear is erect and usually about 9m to 18 m or more in height and the trunk may be up to 30 to 60m in diameter (Owolabi *et al*., 2010). The huge cost of managing hypertension and the low income of people make the management of hypertension difficult and unaffordable for people in developing countries such as Nigeria, thus making a lot of people vulnerable to HBP induced organ damage, complication and mortalities. However, an alternative can be found in *Persea americana*, in the management of cardiovascular conditions because of its easyavailability and low cost.

1.1 Statement of Problems

Cardiovascular disease (CVD) is currently a great healthcare challenge in the world. It is common in high, middle and low-income countries. Several sub-Saharan countries are currently facing severe and multiple health challenges stemming from rising cases of metabolic and non-infectious diseases amidst the high prevalence of other diseases. In addition, the economic effects at the level of each household and macro-economic level are quite appreciable due to exorbitant` and catastrophic healthcare expenditure, loss of income and labour productivity (WHO, 2013). Despite the fact that the treatment of affected individuals reduce CVD pathology and death (Law *et al*., 2009), there is still low coverage of treatment of patients at risk in sub-saharan Africa countries. (Law *et al*., 2009,). This problem persists because of dysfunctional or moribund healthcare delivery system as well as lack of affordability.It is worsened by the fact that a significant portion of cost are borne by the patients (Samb *et al*., 2010).

Oxidative stress was reported to be paramount on the pathophysiologic mechanisms of CVDs. Free radicals have been incriminated in the development of disease conditions like atherosclerosis, ischemia-reperfusion injury, myocardial infarction, cardiomyopathy, congestive heart failure and diabetes mellitus(Zalba*et al*., 2001). Current available scientific information established the relationship between ROS and cardiovascular organ damage, vascular tissue injury, hypertension and dyslipidemia. This knowledge can be utilized in formulating effective clinical and pharmacological techniques for intervention and therefore, medicinal plants that possess the ability to mitigate the activation of signaling pathways that could initiate, propagate and

maintain the processes that could lead to cardiovascular diseases will be viable candidates for investigation for cardiovascular therapy.

1.2 Justification of the study

In African countries including Nigeria, several medicinal plants exist which have been used for ages in the management of diseases. *Persea americana*(PA) and some other plants were reported to have anti-hypertensive and antioxidant activities. However, despite the abundance of these natural resources in Nigeria, there is a dearth of information on their possible uses in the prevention, amelioration and treatment of CVD which are mediated by oxidative stress. Identification and deployment of these plants in the management of CVD, owing to their reported anti-oxidative properties would go a long way in relieving the healthcare system which is already overburdened by pre-existing communicable diseases. It would also reduce the cost of managing or treating hypertension. Most anti-hypertensives have been found to have side effects, hence the need to get an alternative in herbs such as *Persea americana* with antihypertensive effect. Therefore the mechanism of action of *Persea americana* in regulating the signaling operation of hypertension needs to be well understood and the toxic constituent would be isolated to make the plant extract safe for use in management of hypertension in humans and animals.

1.3 Aims

To study the therapeutic efficacy of *Persea americana* leaf, bark and root extracts and their modes of action.

1.4 Objectives

1. To evaluate the in-vitro antioxidant, anti-inflammatory and anti-hypertensive activities of PA extracts and fractions.
2. To carry out phytochemical analyses *P. americana extracts*and determine safety.
3. To evaluate the anti-hypertensive properties and mechanismsof action of *PA* in hypertension in rat.
4. To determine the effect of treatment with PA on electrocardiographic parameters of hypertensive rats

5. To isolate the bioactive phytocompounds in the methanol leaves, bark and root extracts in *P. americana.*
6. To evaluate the in-vitro biological antioxidant, anti- inflammatory and anti-hypertensive activities of the isolated pure metabolites.

1.5 Study Hypothesis

i. Ho 1 : The leaves, stem bark and root of Persea americana are not toxic in rats

ii. Ho2: The leaves, stem bark and root of *Persea americana* extracts do neither have anti-hypertensive effect nor protect against hypertension-induced organ damage.

iii. Ho3: The leaves, stem bark and root of *Persea americana* extracts do not possess antioxidant and anti-inflammatory activities.

iv. Ho4: Leaves, stem bark and root of *Persea americana* extracts cannot inhibit angiotensin converting enzyme

Null Hypothesis 1: To accept Hypothesis

CHAPTER TWO

LITERATURE REVIEW

2.1 HYPERTENSION

Hypertension (HBP) is a state of persistent elevation of arterial blood pressure (Nandhini, 2014). Blood pressure (BP) measurement and reading comprise of systolic and diastolic pressure, which measure the BP on the contraction (systole) or relaxation (diastole) of the heart (Nandhini, 2014). Hypertension is confirmed in humans and rats when the systolic blood pressure is greater than 140 (>140) mmHg and/or diastolic blood pressure is greater than 90 (> 90) mmHg. It is a global health challenge of great concern (Weber *et al*., 2014) and it predisposes patients to other cardiovascular diseases (Go, 2013). Countries in the sub-Saharan Africa (SSAs) are currently faced with multiple health challenges stemming from a rising prevalence of cardiovascular diseases amidst the high prevalence of pre-existing communicable diseases (WHO, 2015). In addition to this, the economic effects at the level of each household and macro-economic levels are quite appreciable due to exorbitant and catastrophic healthcare expenditure, loss of income and labour productivity (WHO, 2015). Although treatment of affected individuals reduces occurrence of CVD and CVD associated death (Law *et al*., 2009),this problem continues to persist because of dysfunctional or moribund healthcare delivery system, lack of affordability and the fact that a significant portion of treatment bills are borne by the patients (Samb *et al*., 2010).

HBP has been reported to be the principal predisposing factor for development of brain infarction, myocardial infarction and renal dysfunction in Nigeria (Ogah *et al*., 2012). Furthermore, its rising prevalence in the country, especially among the adult population, may soon result in remarkable economic and health challenges if not checked (Ogah *et al*, 2012). In companion animals, especially dogs and cats, CVD, expecially hypertension, is a significant cause of morbidity and mortality. It usually

occurs secondary to an underlying disease with resultant organ dysfunction. The target organs usually affected include the nervous system, the kidneys, the eyes and the heart. Hypertension has also been reported in canine babesiosis, a blood parasite infection of dogs prevalent in Nigeria (Omobowale and Nottidge, 2011).

The predisposing factors of HBP includeobesity, high salt consumption, intake of excessive alcohol and sedentary lifestyle (Ogden *et al*., 2000). As a result, much has been invested on the awareness, management and treatment but despite this, approximately 7.1 million annual death has been attributed to hypertension (Chobanian *et al*., 2003).

2.2 PATHOPHYSIOLOGY OF HYPERTENSION

Hypertension can be mainly and broadly categorised into essential or idiopathic and secondary (Delacroix *et al*., 2014). Essential hypertension accounts for the bulk (95%) of hypertensive patients (Delacroix *et al*., 2014; Weber *et al*., 2014). Although the cause of essential hypertension is unknown, it is usually associated with lifestyle disorders such as: excessive salt intake, obesity, reduced physical activity, excess alcohol intake e.t.c (Ogden *et al*., 2000). There can also be a genetic predisposition to hypertension (Weber *et al*., 2014). Conversely, secondary hypertension is mostly associated with underlying medical conditions such as renal and endocrine diseases and nervous disorders (Weber *et al*., 2014). A small percentage (2-5%) of patients diagnosed for hypertension had underlying renal and adrenal disorders (Gareth *et al*., 2001). However, what is common to both classes of hypertension is they are both associated with a derangement of mechanisms by which the BP is maintained in man and animal. (Beevers *et al*., 2001).

Cardiac output, peripheral resistance, endothelial dysfunction, autonomic nervous system, renin-angiotensin-aldosterone system are involved in the maintenance of blood pressure.

2.2.1 Cardiac Output and Peripheral Resistance

The normal BP of animal is maintained by the equilibrium of cardiac output (CO) and resistance of the peripheral blood vessels (PVR) (Delacroix *et al*., 2014). It was reported that most patients with essential hypertension had normal CO but elevated vascular resistance (Gareth *et al*., 2001). Peripheral resistance is dependent on the

contracting effect of intracellular calcium on the vascular myosite of small arterioles. Prolonged smooth muscle contraction causes arterial musculature remodelling and persistent rise in the peripheral resistance (Gareth *et al*., 2001).

On the onset of hypertension, the elevation of blood pressure is brought about by the initial hyperactivity of the sympathetic nervous system which ultimately results in increased cardiac output. (Mayet and Hughes, 2003). This causes a rise in peripheral arteriolar resistance to protect the capillaries and maintain cell homeostasis (Gareth *et al*., 2001; Mayet and Hughes, 2003).

2.2.2 Endothelial Dysfunction

Various researchers elucidated the role of endothelial dysfunction in hypertension development (Panza *et al*., 1995). The severity of hypertension is dependent on the extent of endothelial pathology (Benjamin *et al*., 2004).A dysfunction in the endothelium has been associated with primary hypertension because the impairment of the endothelium affects the potent vasoactive agents produced by the endothelium. The vasoactive agents regulate the blood pressure and alteration in their production affects their functions (Delacroix *et al*., 2014). These agents include nitric oxide, angiotensin II, endothelin, bradykinin, and several other cells proliferation / growth factors (Gareth *et al*., 2001 & Dreishbach, 2015). An alteration in the normalcy of the endothelium will result in the disruption of the secretion of these vasoactive agents (Dreishbach, 2015).

Hypertension is associated with increased production of reactive oxygen species and free radicals. These result in oxidative stress and decreased the bioavailability of endothelial nitric oxide, vascular remodelling and hypertension (Sander *et al*., 1999).

Inhibition of nitric oxide synthase has been reported in hypertensive patients thus confirming the fact that a reduced bioavailability of endothelium derived nitric oxide and dysfunction of the endothelium play important role in the development of hypertension (Sander *et al*., 1999). Nitric oxide (NO) plays a significant role in dilation of blood vessels and regulation of funtions of associated vital organs. (Laursen *et al*., 1997). NO also regulated the proliferation and functions of platelets, it responds to nervous control of homeostasis and ultimately play a role in regulation of blood pressure, intravascular blood coagulation, thrombus formation and vascular

remodelling (Laursen *et al*., 1997). Some medications initiate the production of nitric oxide in hypertensive patients but the damage on the endothelium is irreversible (Sander *et al*., 1999). Therefore the endothelium will not respond to endothelial agonist (Gareth *et al*., 2001).

NO and other vasorelaxing agents play significant roles in vascular tone maintenance.(Delacroix *et al*., 2014). Oxidative stress and vascular inflammation have been reported to be a major cause of endothelial dysfunction (Coca-Robinot *et al*., 2005). Conversion of Angiotensin I to Angiotensin II and inactivation of bradykinin, a potent vasodilatoris done by Angiotensin converting enzyme (Gareth *et al*., 2001).

Angiotensin II has been discovered to cause elevation of blood pressure by vasocontraction effect and enhancement of the production of the superoxide anion at a low concentration (Laursen *et al*., 1997). Endothelin also play roles in the pathogenesis of hypertension by its vascular constriction effect and potentiation of angiotensin –aldosteron system leading to production of angiotensin II (Gareth *et al*., 2001, Dreishbach, 2015).

2.2.3 Renin-Angiotensin-Aldosterone System

This system is regulated by the kidney and the brain and it controls majorly the intravascular and extracellular fluid and the resistance offered by the blood vessel against the circulating blood. It regulates electrolyte balance and may also contribute to the development of HBP (Lahogue *et al*., 2010). Renin is produced principally by the kidney and it is the hormone that initiate the process that result in production of Angiotensin (Saxena et al., 1992). Renal dysfunction has been suggested by substantial data to be an underlying factor in the pathophysiology of all kinds of HBP. Renal dysfunction may be secondary to the derangements in body functions like endocrine, nervous and vascular abnormality resulting in impairment in sodium excretion, increase intravascular pressure and pathology of the kidney ultra-filteration apparatus. (Dreisbach, 2015). Also, the activities of the renin-angiotensin-aldosterone system influence the progression of renal disease (Dreisbach, 2015).

Renin is produced and released from the juxtaglomerular apparatus cells in the kidney when there is hypotention.The production is stimulated by the sympathetic impulses

(Sarsani *et al*., 2008). Renin cleaves circulating angiotensinogen to angiotensin I, a relatively inactive substance, in the blood stream which is then cleaved to a very potent vasoconstrictor, angiotensin II (Piepho and Beal, 2000). The angiotensin II is circulated in the blood to the target organs, including the adrenal glands, blood vessels and kidneys. It binds to its specific receptors where it elicits its actions, thus, contributing to aldosterogenesis (Piepho and Beal, 2000). Angiotensin II acts on angiotensin I receptors to enhance the synthesis and release of aldosterone, which inturn act on the proximal convoluted tubules of the nephrons to excrete potassium and hydrogen ions into the tubules. It also increases Na+ reabsorption thereby increasing blood pressure (Dreisbach, 2015).

2.2.4 Autonomic Nervous System (ANS)

Various scientific studies have shown that the pathophysiology of hypertension can result from sympathetic stimulation of the heart and associated organs causing tachycardia, increased cardiac output, intravascular volume and vascular resistance (Gareth *et al*., 2001). This can result in sustained increase in blood pressure (Gareth *et al*., 2001). Alteration in the balance in ANS are caused by metabolic haemodynamic and endocrine disorders which altimately result in cardiovascular disease, associated complications and death (Brook *et al.*, 2000).

The role of epinephrine (adrenaline) and norepinephrine (noradrenaline) in the aetiology of hypertension is not clear, but the sympathetic receptor blockers reduce heart rate and blood pressure and are invariably used in the management of hypertension (Gareth *et al.,* 2001).

Persistent stimulation of the sympathetic nervous system have been associated with vascular –remodelling, and ventricular hypertrophy through direct action of the nor-epinephrine (Brook *et al*., 2000). Thus, autonomic nervous system contributes to the pathogenesis of hypertension and the development of target organ damage. The importance of the interaction between ANS, the renin-angiotensin system, and other factors such as electrolyte balance, circulating and hormonal disorders cannot be over emphasized (Gareth *et al*., 2001).

2.3 TYPES OF HYPERTENSION

2.3.1 Primary Hypertension

Essential hypertension forms the bulk (95%) of hypertension cases (Weber*et al*., 2014). It is usually attributed to the interaction between hereditary and environmental factors (Carretero and Oparil, 2000; Nandhini, 2014) with majority of cases being caused as a result of life style disorders such as excessive salt intake, obesity, reduced physical activity, excess alcohol intake e.t.c (Ogden *et al*., 2000). The prevalence of essential hypertension increases as the animal advances in age (Ogden *et al*., 2000).

Blood pressure elevations at younger ages have been correlated with development of hypertension in individuals at older ages (Ogden *et al*., 2000). Essential hypertension may also be caused by Intra-uterine malnutrition, low dietary calcium, magnesium and potassium, excessive drug use, emotional stress, diet, pill, oral contraceptives among others (Carretero and Oparil, 2000).

2.3.2 Secondary Hypertension

Secondary hypertension accounts for 5% of hypertension cases (Delacroix *et al*., 2014). Secondary hypertension is usually associated with a pre-existing medical conditions that directly or indirectly result in hypertension (Carretero and Oparil, 2000). Diseases associated with secondary hypertension include chronic renal failure, diabetes, coarctation of the aorta, or disorders of the endocrine system such as hyperaldosteronism, hyperthyroidism and hyperadrenocorticism. (Carretero and Oparil, 2000).

2.4 MANAGEMENT OF HYPERTENSION

Hypertension is managed by using different types of drugs, one of these is Angiotensin converting enzyme inhibitors (ACEI). ACEI acts on ACE sites in tissues to reduce high blood pressure by exerting cardio and reno-protective activities (Dzau *et al*., 2001). ACEI prevents angiotensin II production and thus lead to reduction in blood pressure (Otte *et al*., 2007). It however enhances the accumulation of bradykinin. Angiotensin II is a growth promoter and it also acts on vascular smooth muscle, it causes vasoconstriction and promotes cellular growth. Bradykinin is a vasodilator, growth inhibitor and antagonist of angiotensin II (Tom *et al*., 2003). ACEI prevents complications in the heart and kidney. (Thurman and Shrier, 2003).

Angiotensin II may be produced by an alternative pathway and it occurs when the level of angiotensin I increases, leading to the formation of angiotensin II (Thurman and Shrier, 2003). Here angiotensin I inhibitors block angiotensin II at angiotensin I receptor sites making it available for angiotensin II receptor to trigger the pathway for bradykinin and nitric oxide (NO) or cyclic guanosine monophosphate cycle (Thurman and Shrier, 2003).

ACE peptide inhibitors were discovered in snake venom and they were recognized and used in hypertension management (Bryan, 2009). Other ACEI were identified e.g. captopril while some others were synthesized later including enalapril and lisinopril (Bryan, 2009).Although these drugs can be used singly, they can also be used in combination with other anti-hypertensive medications such as calcium channel blockers (CCB) and diuretics (Hollenberg, 2003).

Lisinopril, has a molecular weight of 441.52. The drug is white in colour and it dissolves easily in water, less so in methanol. Ethanol however can not be used as a sovent for it because it does not dissolve in ethanol. It is administered orally ans has a long duration of action. It is an oral long acting ACEI, a lysine equivalent of analaprilate (an enalapril metabolic product) (Makipour *et al*., 2008). Lisinopril as other ACEI controls hypertension, especially the essential type and reduces cardiovascular risk by causing the build up of bradykinnin in biological fluids and by obstructing the renin-angiotensin system (Makipour *et al*., 2008). Lisinopril is well tolerated in controlled clinical trials but when given at high doses in patients with cognitive heart failure, results in hypotensive symptoms like syncope and dizziness, impaired renal function with increased serum creatinine and hyperkalaemia. Side effects like cough, neutropenia, skin rashes, angioneurotic oedema, headache and dizziness are also associated with lisinopril administration (Makipour *et al*., 2008). Lisinopril also causes anaemia (Makipour *et al*., 2008).

Angiotensin receptor blockers (ARBs) act the same way like the ACEIs in that they also target the renin-aldosterone-angiotensin-system and act by blocking the AT1 receptors that are responsible for angiotensin II function (Delacroix *et al*., 2014). Both the ARBs and ACEIs can be combined for better result. An added advantage of using the ARBs is that side effects such as coughing that is observed with ACEIs are absent (Delacroix *et al*., 2014).

Calcium channel blockers inhibit vascular smooth muscle cells and cardiac myocytes contraction by blocking L-channels in these cells; thus inhibiting the influx of calcium ions (Costanzo *et al*., 2009). Dihydropyridines are vasodilators and cause vasodilation and decrease in peripheral vascular resistance (Costanzo *et al*., 2009). Non-dihydropyridines on the other hand act by reducing both the heart rate and myocardial contractile force (Costanzo *et al*., 2009).

Diuretics reduce blood pressure through the excretion of sodium ions and water (Hollenberg, 2003). It has been observed that even small doses of diuretics potentiate the antihypertensive effects of other drugs when used in combination with them (Delacroix *et al*., 2014).

Other classes of antihypertensive drugs include: beta blockers, direct vasodilators and direct renin inhibitors (Sanoski, 2009).

It is however worthy of note that, lifestyle changes such as reduced alcohol consumption, regular excercise, reduced dietary salt intake, weight reduction and caesation of smoking in conjuction with pharmacologic agents in subjects with hypertension (Elmer *et al*., 2006).

2.5 OXIDATIVE STRESS AND HYPERTENSION

Oxidative stress plays significant role in the development and pathology of various forms of CVD (Baradaran *et al*., 2014). In oxidative stress, there is an alteration in the equilibrium of the oxidant and anti-oxidant systems leading to an increase in the formation of Reactive Nitrogen Species (RNS) and Reactive Oxygen Species (ROS) both of which are harmful to the cells (Tuteja *et al*., 2001). The molecule NO, endothelium relaxant factor produced by endothelium nitric oxide synthase (eNOS), is essential in vascular homeostasis as a modulator of endothelial tone and reactivity and it is known to exert pleiotropic positive effects on the cardiovascular system (Martindale and Holbrook, 2002). Under heightened cellular oxidative stress conditions, NO bioavailability is greatly reduced as generated free radicals combine with NO to produce peroxynitrite, which further causes an aggravation of the already existing oxidative imbalance. The generation of ROS is known to activate molecules like nuclear factor kappa β [NF-κβ] (Martindale and Holbrook, 2002), which has

beenimplicated in many organs dysfunction involving the liver, heart, lungs, and kidney (Yousefipour *et al*., 2007). Antioxidants can protect not only against direct free radical toxicity and inhibit the activation of NF-κβ and its subsequent damages (Suzuki *et al*., 1997). Since ROS have the capacity to bring about vascular cell and cardiomyocyte damage, their increased production is therefore linked with the aetiology and pathogenesis of atherosclerosis and cardiomyopathy in humans.

ROS also have the ability to trigger a number of hypertrophy signalling and transcription factors (Sabri *et al*., 2003).

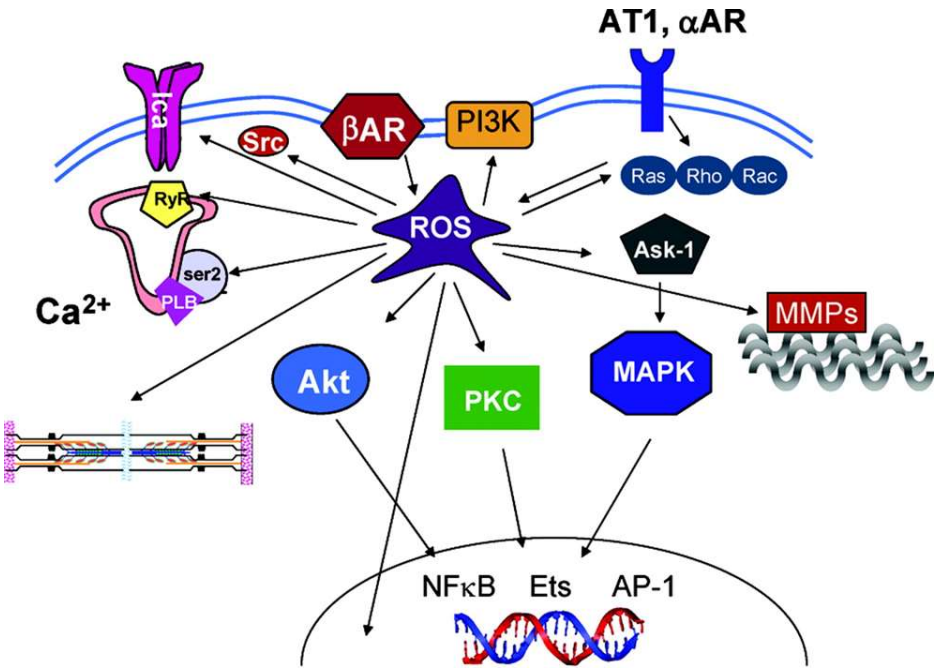


Figure 2.1 Mechanism of ROS induced cardiac remodelling and hypertrophy.(Takimoto and Kass, 2007)

2.6 MARKERS OF OXIDATIVE STRESS AND OTHER BIOLOGICAL MARKERS

Nitric oxide is colourless and regarded as a free radical since it possesses an unpaired electron (Santo *et al*., 2016). Nitric oxide (NO·), nitrogen dioxide (NO_2·), and peroxynitrite (OONO−) are the notable reactive nitrogen species (Phaniendra *etal*., 2015). Peroxynitrite is a product of nitric oxide and supoxide reaction. It is a powerful oxidant compound which can induce cellular necrosis and apoptosis (Pacher *et al*., 2007). Build up of ROS in the blood vessel wall results in decrease in the endothelial NO and this ultimately alters the function of the vascular smooth muscle. (Lee *et al*., 2012).

2.6.1 Myeloperoxidase

Myeloperoxidase is an enzyme produced by neutrophils during phygocytosis and it is essencial in body innate response to antigen and inflammation (Loria *et al*., 2008). Myeloperoxidase is ablood cell protein that is found in the azurophilic granules of polymorphonuclear cells and monocytes (Kolaczkowska and Kubes, 2013). Myeloperoxidase catalysis the production of hypochlorous acid from hydrogen peroxide and chlorine during phagocytosis and cellular inflammatory processes (Heinecke, 1999).

2.6.2 Glutathione, Glutathione Peroxidase and Glutathione S-Transferase

Glutathione in its reduced form is an essential antioxidant in the cells. It protects the body cells from a wide varirties of reactive oxygen species (Masella *et al*., 2005). The mechanisms of action of glutathione as antioxidant are diverse (Masella *et al*., 2005). GSH scavenges free radicals and ROS, such as, hydrogen peroxide, hydroxyl radical and so on by enzymatic and non-enzymatic reactions (Santo *et al*., 2016). In its antioxidant activities, GSH is oxidized to glutathione disulphide. (GSSG) (Santo *et al*., 2016).GSSG is in turn reduced to GSH by the enzyme glutathione reductase and NADPH(an electron donor) as high concentrations of GSSG can destroy many enzymes through oxidative damage (Wu *et al*., 2004). Glutathione Peroxidase (GPx)

catalysis the detoxification of hydrogen peroxide and lipid peroxides by GSH (Birben *et al*., 2012). Glutathione reduces hydrogen peroxide to H_2O and O_2 by donation of an electron (Birben *et al*., 2012). These isozymes GPX catalyse the reduction of hydrogen peroxide to water using reduced glutathione as the reductant. GSH is oxidized to GSSG in the process and is later converted back to GSH by the enzyme glutathione reductase (Wu *et al*., 2004). Glutathione S-transferases (GSTs) belong to the phase 2 enzymes that protect cellular macromolecules from the deleterious effects of electrophilic compounds within and around the cells (Townsend and Tew, 2003). This family of enzymes perform this function by catalysing the conjugation of glutathione to reactive oxygen species (Townsend and Tew, 2003).

2.6.3 Hydrogen Peroxide and Catalase

Hydrogen peroxide (H_2O_2) is a biological ROS which is harmful to the cells and tissues when in excessive amount (Santo *et al*, 2016). The H_2O_2 is a by-product of respiration and the end-product of many metabolic processes with the major site of production being the peroxisomes (Cheeseman and Slater, 1993). Hydrogen peroxide on its own is a very weak oxidant but it has the ability to produce a more reactive product such as the hydroxyl (OH^-) radicals especially through the Haber-Weiss reaction (Winterbourn, 2013). This weakness is because the oxygen to oxygen bond of the H_2O_2 is very weak and very prone to cleavage by various external agents such as ionizing radiation and ultraviolet rays (Winterbourn, 2013).

Catalase, an antioxidant enzyme is found primarily in the peroxisomes, catalysis the breakdown of $H_2O_2$to non-toxic oxygen and water (Kodydkova*et al*., 2014).

$$(2\ H_2O_2 \rightarrow 2\ H_2O + O_2)$$

Catalase therefore prevents the build-up of H_2O_2 within the cells thus preventing the subsequent production of the more reactive OH^- radical which is lethal to body cells and tissues (Kodydkova*et al*., 2014; Santo *et al*., 2016).

2.6.4 Superoxide Dismutase

Superoxide dismutase (SOD) enzymes play vital roles in enzymatic dismutation of superoxide into hydrogen peroxide and molecular oxygen (Mruk *et al*., 2002).

$$(2\ O_2^{\cdot -} + 2\ H+ \rightarrow H_2O_2 + O_2)$$

SODs are characterised by their possession of transition metals at their active sites and depending on the type of transition metals, they are grouped into three: copper, zinc superoxide dismutase (SOD 1), manganese superoxide dismutase (SOD 2) and extracellular superoxide dismutase (SOD 3). These superoxide dismutases are present in varying locations in the cells, for instance with SOD 1 is found in the cytoplasm, SOD 2 in the mitochondria. SOD 3 is extracellular (Buettner, 2011).

2.7PERSIA AMERICANA

Persea americana, commonly known as avocado is an almost evergreen flowering fruit tree. There are about 60 different varieties which occurs probably due to diversity of environmental conditions or human intervention. Avocado was first grown in South Central Mexico but is been commercially cultivated in countries with tropical and Mediterranean climates across the globe (Morton, 1987). Some of these varieties include the West Indian avocado, Mexican avocado (*P.americana mill var drymifolia blake*),Guatemalan avocado (*P.nubigena var guatemalensis*),Hass cultivar, Lamb Hass, Reed, Pinkerton, Bacon, Walter Hole, Fuerte, Sharwil, Zutano, etc.

Depending on the cultivar and variety, the tree is erect with height usually about 30ft but could be as high as 60ft high, a trunk of about 12-24 inches in diameter (Morton, 1987).

The fruits have smooth or rough skin with variable colours from yellow-green to purple fleshy and the shape may be pear, egg-like or spherical. It's about 7-20cm long and weighs between 100and 1000g.The fruit contains a large central seed of about 5-6.4 cm (2.0-2.5in) long (Morton, 1987).

The leaves are alternate in arrangement, dark green colour and glossy on the upper surface. The under surface of the leaves is whitish and of variable shapes. The shapes vary from lanceolate, elliptic, oval, ovate to obovate and they are 7.5 – 40 cm long (Morton, 1987).

The flowers are not so conspicuous, they are greenish-yellow in colour and 5–10 mm wide. (Morton, 1987).There are two flowering types: Winter flowering and Spring flowering.

These flowering pattern differences are based on different cultivar. The flowers of cultivar A (e.g.'Choquette, Hass, lula, maluma etc.) open as female in the morning of

the first day and close in late morning or early afternoon. Then they open as male in the afternoon of the second day. The flowers of cultivar B varieties (e.g. Bacon, Brogden, Monreo etc.) open as female on the afternoon of the first day, close in late afternoon and reopen as male the following morningas a result of their breeding pattern (Edward *et al*., 1994).

Scientific Name: *Persea 19mericana*
Genus: *Persea*
Species: *19mericanaMill*

(United States Department of Agriculture. National Resources Conservation Service. Classification. April, 2012.)

2.7.1 Medicinal Value and Other Uses

Various research works have shown that the aqueous leaves extract has capability to reduce pain and inflammation (Ojewole and Amabeoku, 2006), It was also reported to possess the ability to reduce the blood glucose and cholesterol levels (Brai *et al*., 2007), it also has blood vessel relaxation and blood pressure reduction effects (Owolabi *et al*., 2010; Ojewole, 2007) in animal studies. The leave extract increases the stored lipid catabolism resulting in weight loss (Brai *et al*., 2007). The leave extract is also used to treat childhood convulsion and epilepsy in African traditional medicine and also has anticonvulsant effect in mice (Ojewole and Amabeoku, 2006). The leaves extracts have been taken orally to treat pyorrhoea, diarrhoea and sore throat (Brai *et al*., 2007).

Avocado is also grown as household plant. The presence of tannin in avocado seed makes it turn red when exposed to air (atmospheric air) hence, its use in document writing and marking of cotton and linen textiles (Morton, 1987).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Objective 1

3.1.1INVITRO ANTI-OXIDANT, ANTI-INFLAMMATORY AND ANTI-HYPERTENSIVE ASSAYS OF PERSEA AMERICANA EXTRACTS.

3.1.1.0 Plant Extraction and Extract Fractionation

Fresh *P.20mericana* leaves were cut, air-dried, blended and soaked in N-hexane for 48hours. This was done to remove the fat in the leaves. The leaves were sieved and re-dried. The re-dried leaves were then soaked in methanol for 72 hours, then squeezed using a cloth sieve to obtain the extract (fluid) from the leaves. Rotary evaporator was used to concentrate the extracted solution and to remove the methanol through evaporation. A greenish (leaf extract) and brownish (stem bark and root extracts) pasty extract were derived and reconstituted with corn oil into a 100mg/ml and 200mg/ml solution.The extracts wee then fractionated by liquid-liquid partitioning in different solvent from non polar to polar solvents including H-Hexane, Ethyl acetate, Chloroform, N-butanol and aqueous methanol using separating funnel. (Abubakar and Haque, 2020)

3.1.1.1 IN VITRO ANTI-OXIDANT ASSAYS

3.1.1.2. 1, 1- Diphenyl – 2 Picrylhydrazyl (DPPH) Radical Scavenging Assay

The anti-oxidant activities of the various extracts of *P. 20mericana* were assayed by quantifying the ability of the extracts to decolourize the purple-coloured DPPH in methanol solution, as described by Turkoglu *et al.* (2007). In this assay, 1 mL of 0.2 mM DPPH methanol solution was added to 1 mL of various concentrations (0.0156-0.250 mg/mL) of the extracts and incubated at 25^0C for 30min. the absorbance of the

resulting mixture was measured at 516 nm using a microplate reader (BIO RAD. Model 680, Japan). Theformula for calculating percentage inhibition rate (1%) is as below.

Percentage inhibition (1%) = $[(A_{control} - A_{extract})/A_{control}] \times 100$

$A_{control}$ = absorbance of thecontrol, $A_{extract}$ =absorbance of the extract.

The concentration of*P. americana* extracts causing 50% inhibition (IC_{50}) of DPPH radical was calculated using thei standard calibration curve.

3.1.1.3 Nitric Oxide Scavenging Ability

The capacity of *P. americana* extracts to scavenge nitric oxide radical was evaluated using the procedure reported by Garrat (1964).Sodium nitroprusside (10 mM) was prepared in 0.5 mL phosphate buffer saline (pH 7.4). Precicely 2mL was mixed with 0.5mL ofextract with varying concentrations (0.0156 - 0.25 mg/mL) with distilled water as control in 10 mLThe mixture wasthen incubated at 25^0C for 2.5 hrs, after which 0.5mL was taken from the incubated mixtures and added to 0.5 mL of 1% sulfanilic acid and 2% H_3PO_4. The mixture was further incubated at 25^0C for 5 mins. Thereafter, 0.5 mL N-(1-naphthyl) ethylenediamine dihydrochloric acid (0.1 % w/v) was added to the solution which was then left on the bench to incubate at room temperature for 30 min. The mixture was read at 540 nm.

Percentage inhibition (1%) = $[(A_c - A_e/A_c] \times 100$

Where $A_c$and A_e denote the readings for control and the extract, respectively. The concentration of*P. americana* extracts causing 50% inhibition (IC_{50}) of NO radical calculated from the standard calibration curve.

3.1.1.4 Metal Chelating Ability of the Extracts

The metal chelation ability of *P. americana* extracts was assayed following the procedure of Dinis *et al*. (1994). Summarily, 0.1 mL of the extract (0.0156 – 0.25 mg/mL) was added to 0.5mL of 0.2mM ferrous chloride solution. The reaction was started by adding 0.2 mL of ferrozine (5mM) and incubating at 25^0Cfor 10 minutes.The absorbance was read at 562 nm in a microplate reader (BIO RAD, Model 680, Japan). Citrate was used to control and the chelating potential of the extracts that

competed with ferrozine for the ferrous ions was revealed from the colour reduction. IC_{50} value was extrapolated from the calibration curve.

3.1.1.5 Reducing Power Property

The reduction property of the extracts was assessed according to the method of Oyaizu (1986). Different concentrations (0.0156- 0.25 mg/mL) of *P. americana* extracts were added to 1mL of distilled water and then mixed with 2.5mL of 0.2 m phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferrocyanide. The mixture was incubated at 50^0C for 20 minutes before the addition of 2.5 mL of trichloroacetic acid. The resulting mixture was centrifuged at 3,000 rpm for 10 minutes after this, 2.5 mL of the supernatant was mixed with an equal amount of distilled water and 0.5 mL of 0.1% $FeCl_3$. The colour change of the resulting solution was then taken at 700 nm.

3.1.1.6 2,2' Azinobis (3-Ethylbenzothiazoline- 6-Sulfonic Acid) ABTS Radical Scavenging Ability

The test was conducted using the procedure of Re *et al*. (1999). The $ABTS^+$ was generated by reacting 7mM ABTS aqueous solution $K_2S_2O_8$ (2.45 mM, final concentration) in the dark for 16 h and adjusting the pH to 0.7 with ethanol. Exactly 0.2 mL of the various dilutions of extracts (0.156 - 0.250 mg/mL) was added to 2.0 mL $ABTS^+$ solution and the absorbance was measured at 734 nm after 15 min. The gallic acid equivalent antiodant capacity was therafter determined.

3.1.1.7 Hydroxyl Radical Inhibitory Potential

The ability of the various extracts of *P.americana* to stop Fe_2^+/ H_2O_2induced decomposition of deoxyribose was assayed using the modified method of Oboh *et al*. (2007). The reaction product was read at 532 nm in a microplate reader (BIO RAD, Model 680, A standard antioxidant, gallic acid, was used as the control at varying concentrations (0.156 – 0.25 mg/mL), and the IC_{50} value was then calculated from the calibration curve.

3.1.2Angiotensin I converting enzyme (ACE) inhibition assay

ACE inhibition assay was performed on the basis method from literature (Oboh *et al*., 2012, Lin and Li, 2012) with little modification The principle was to evaluate the inhibitory activity of extracts, fractions, test compounds and captopril on the ACE enzymatic conversion of 6.5mM of hippuryl-histidyl-leucine (HHL) to produce Bz-Gly (hippuric acid) which was extracted by ethyl acetatate in form of acetate supernatant following centrifugation at 4000 rpm for 15 minutes. The ethyl acetate was removed by heat evaporation and the residue dissolved in 3mL volume of distilled water. Absorbance was read at 228 nm using a spectrophotometer (UNICO UV-2102, Shanghai, China). .

Calculation of percentage inhibition.

$$Inhibition\ (\%) = \left(\frac{A_a - A_b)}{A_a - A_C}\right) X\ 100$$

Where A_a is the absorbance with ACE and HHL without the sample (positive control);

A_b is the absorbance with ACE, HHL and the sample or standard; and A_c is the absorbance with HHL without ACE and the sample (control). IC50 was calculated by IC 50 calibration curve.

3.1.3 *In-vitro* Anti-inflammatory Assay.

The anti-inflammatory activities was evaluated by the *invitro*15-lipoxygenase enzyme inhibition assay described by Adebayo *et al*. (2015)

The 15-LOX (Sigma) inhibition assay is to measure the inhibitory activities of the extracts, fractions and pure compounds relative to that of standard flavonoid, quercetin and indomethacin, a standard NSAID. Lypoxygenases are responsible for the conversion of linoleic acid to prostaglandin, a proinflammation product.

The absorbance was measured with the microplate reader at 234 nm (SpectraMax 190, Molecular devices). Quercetin (1 mg/mL) was used as a positive control, while DMSO was used as the negative control (100 % enzyme activity or no enzyme inhibition).

$$\%\ \text{Inhibition} = \frac{\text{OD extract - OD blank X100\%}}{\text{OD negative control - OD blank}}$$

The results were expressed as IC50, i.e. concentration of the extracts and controls that resulted in 50 % 15-LOX inhibition plotted on a graph.

3.1.4 IC 50 Calibration Curve

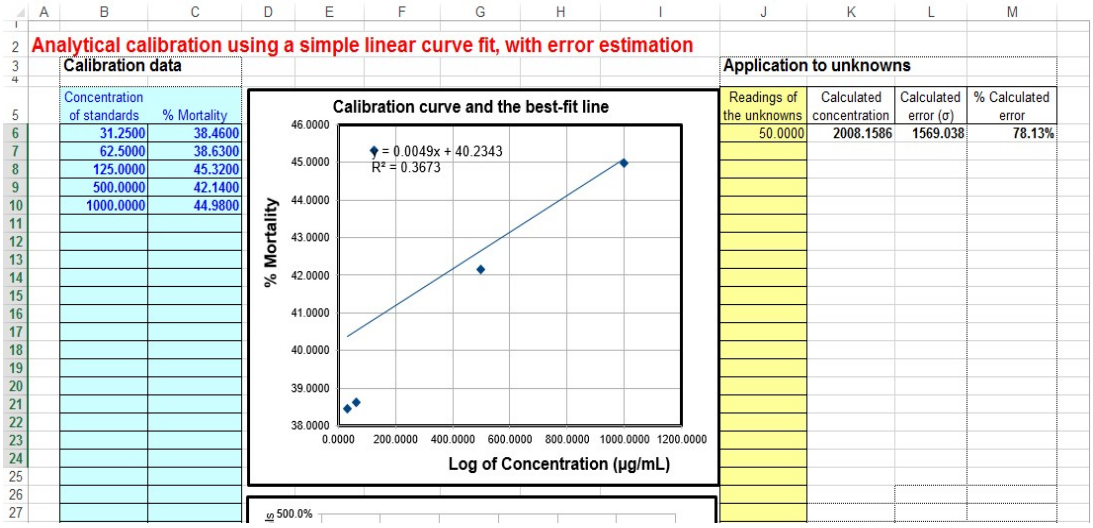


Figure 3.1.1 Showing the IC50 calibration curve.

Figure 3.3.2: Micropipette plates and test tubes used for *in vitro* biological assays.

Figure 3.3.3. Micropipette plates and test tubes used for *in vitro* biological assays and Spectrophotometer being used for taking readings of the reaction products at specified wavelength.

3.2 OBJECTIVE 2

3.2.0 Phytochemical and Acute Toxicity Studies on *Persea americana* Leaf, Bark and Root Extracts.

3.2.1 Plant Extraction

Plant extraction was done as described in objective 1

3.2.2 Experimental Animals

Eighty fourmale Wistar rats, 6 months old and weight ranging from 140-185g were purchased for these studies. The rats were randomly selected and acclimatized to the feed and the environment for about three weeks. Standard rat feed and water *ad libitum* were supplied throughout the period of the experiment. Conducive space with good and adequate ventilation was maintained. Day and night duration of 12 hours were maintained daily throughout the period of acclimatization.

3.2.3 Chemicals and Reagents.

Analytical grade of Methanol, N-Hexaneand the other chemical reagents were utilized for the research work.

3.2.4. Acute Toxicity Study Design.

The toxicity models described by Lorke, (1983) was employed in this study.

From the eighty four Wistar rats used for this experiment, twenty eight ratsallotted to seven groups (A - G) of four rats per group were used separately for studies on PA leaf, stem back and root. For each experiment,Group A was the control while groups B-G received respective dose of the PA extracts 1000, 2000, 4000, 5000, 6000 and 7500 mg/kg. A single dose administration of the extract constituted at the concentration of 200 mg/ml was given to each rat. Treated rats were then observed for behavioral changes within 72 hours post PA administration.

Median lethal dose calculation was done using the formular described by Olukunle *et al* 2014. LD_{50} is calculated using the formular below.

$LD_{50} = \sqrt{M} \times \sqrt{N}$.

Where M is the maximum non-fatal dose and N is the lowest fatal dose.

Table 3.1 Average body weight of the experimental animals premedication and the study design of rats in acute toxicity study of *Persea americana*leaf.

GROUP	AVERAGE BODY WEIGHT	MEDICATION
A (Control group)	100g	Water and feed
B	113g	500 mg/kg of extract
C	103g	1000 mg/kg of extract
D	107g	2000 mg/kg of extract
E	110g	3000 mg/kg of extract
F	107g	4000 mg/kg of extract
G	95g	5000 mg/kg of extract

3.2.5 Blood Pressure Measurement and Electrocardiogram

Following oral treatment with N^{Ω}-Nitro-L-Arginine Methyl Ester (L-NAME), lisinopril and extract, the blood pressure of each rat was measured in conscious rats by tail plethysmography using electrosphyngomanometer (CODA, Kent Scientific, U.S.A). An average of five readings were taken and recorded in the calm state following familiarisation of the rats. The rats were anesthesized using 0.1 ml each of xylazine and ketamine to ensure proper restraint for electrocardiography. The electrocardiogram was taken for 1 minute for each rat to ensure stabilization and proper recording of the electrocardiogram.

3.2.6 Blood Collection and preparation of Serum for Serum Chemistry

Blood was collected from each rat viaretro-orbital vein just caudal to the eyes on the median side using sterile capillary tubes. Dry clean plain bottles and EDTA bottles were used to collect the blood sample. The blood collected in the EDTA bottle was gently rocked and kept for hematological studies. The blood collected into clean plain tubes were placed slanted on the rack to coagulate. Thereafter the serum was separated by centrifuging the sample in sample bottle at 4,000 rpm for 10 minutesat room temperature. Serum yield was refrigerated at -4^{0}C and was laterused for biochemical analysis.

3.2.7 HEAMATOLOGY

Heamatological parameters were measured using the methods described by Linne and Ringstrud, (1999)

3.2.7.1 Red Blood Cell Parameters

3.2.7.1.1 Packed Cell Volume (PCV)

The PCV was measured as the percentage of red cells in the blood volume. Blood was collected into a capillary tube and spun to separate the red cell from the plasma and the PCV was read.

3.2.7.1.2Red Blood Cell (RBC) Count.

The RBC count was done with the aid of the improved Neubaeurheamocytometer method. Blood was drawn to the 0.5 mark on the RBC dilution pipette. RBC dilution fluid was aspirated and added to the level of 101 markon the pipette (1:200) dilution.

The diluted blood was introduced into the heamocytometer and allowed to flow under the cover slip by capillary action.

Using x10 of a high dry objective light microscope, cells in the small 5 squares of the haemocytometer were focused and counted. RBC count per microliter was calculated as a product of the number of cells counted and the dilution factor (200) divided by 1/5 x volume (0.1) or simply, number of cells multiplied by 10,000.

3.2.7.1.3 Haemoglobin Concentration

The Haemoglobin concentration was estimated based on the principle of light intensity using the spectrophotometer. The method employed was the heamoglobincyanide method (HiCN) using a 1ml pipette, 0.02ml of blood which was carefully transferred into 4ml Drabkin's solution in a clean test tube. The tube was covered and the mixture was thoroughly mixed. The tube was kept in a rack to stand for 30 minutes at room temperature.to ensure complete conversion to cyanmethaemoglobin (HiCN). The solution was then poured into a cuvette and the spectrophotometer set at 100% transmittance at 540nm using Drabkin's solution as blank.

3.2.7.1.4Mean Corpuscular Volume (MCV)

MCV is the average volume of a red blood cells in a living animal. It was determined directly using an automated haematology analyzer, here, it was calculated from the PCV and RBC count. Thus:

$$MCV = \frac{PCV\ (\%)}{RBC(X10^6/\mu L)} X\ 10$$

Values were expressed in fentoliters (fL).

3.2.7.1.5 Mean Corpuscular Haemoglobin Concentration (MCHC)

MCHC refers to the percentage of heamoglobin in 1 dL of packed red blood cell corpuscle. This represents the ratio of hemoglobin mass to the volume of red blood cells. It is calculated thus;

$$MCHC = \frac{Haemoglobin\ concentration\ (g/dL)}{PCV\ (\%)} \times 100$$

Values are expressed in g/dl or percentage.

3.2.7.2 WHITE BLOOD CELL (WBC) PARAMETERS

The white blood cell counts and differentials were carried out using the methods described by Linne and Rinsgrud, (1999)

3.2.7.2.1 Total White Blood Cell Count

This was determined using the improved Hawksley haemocytometer and Turk's solution as the white blood cell diluents. Procedure is as described for Red blood cell count above.

3.2.7.2.2 Differential Leucocyte Count

Using the light microscope and the immersion objective lens 200, leucocytes from the Giemsa-stained blood smear were counted from randomly selected fields using the tally counter. They were then categorized into types and the percentage of each type was calculated.

3.2.7.2.3 Absolute Leukocyte Count

This was derived from the total WBC count and the comparative count of every differential cell count expressed as percentage.

3.2.7.2.4 Platelet Counts

This was carried out using 1% ammonium oxalate as diluent. To prepare Ammonium oxalate, 1g ammonium oxalate was measured into 100 ml deionised water.

The lithium heparinized blood sample was mixed thoroughly by rocking the sample bottles to achieve uniform distribution of blood cells. 20 µl of blood was then added to1.98ml of the diluents to obtain a dilution ratio of 1:100. The mixture was then left for 30 minutes to be mixed at an interval of two minutes. Using the Pasteur pipette, the counting chamber was filled and placed in a moist chamber. The chamber was kept on the bench for 20 minutes. Thereafter, the platelets count as platelet /litre was obtained as a product of cell count in 1 mm^2 chamber and 10^5.

3.2.7.3 SERUM BIOCHEMISTRY

Serum analytes including creatinine, blood urea nitrogen, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured using the protocol described by the manufacturer of the kit used (Randox Ltd, UK)

3.2.7.3.1 SERUM CREATININE ESTIMATION

3.2.7.3.1.1Principle.

Creatinine is measured by quatifying the amount of complex formed when it is reacted with picric acid. The complex formed has measurable colour change which was measured with UV Spectrophotometer.

3.2.7.3.1.2 Sample Collection and Preparation

Serum: Stable for 7 days at +2 to +8^{0}C.

Contents	Initial concentration of Solution
CAL. Standard	
RIa. Picric Acid	35mmol/1
RIb. Sodium Hydroxide	0.32mol/1

3.2.7.3.1.3Procedure

Wavelength	492(490-510nm)
Cuvette	1cm light path
Temperature	25/30/37^{0}C
Measurement	Against air

Pipette in cuvette

Working reagent	Standard	Sample
Standard Solution	1.0ml	1.0ml
Sample	0.1ml	-

The reagents are mixed. The absorbance A_1 and A_2 of the standard and samples were read at 30 seconds and 2 minutes of reaction respectively.

3.2.7.3.1.4Calculation

$A_2 - A_1 = \Delta A$ sample or Δ standard

$\frac{\Delta A \text{ sample}}{\Delta A \text{ standard}}$ x standard conc(mmol/1) = µmol/1

3.2.7.3.2SERUM UREA ESTIMATION

The estimation was carried out using the Randox kit.

3.2.7.3.2.1 Principle

Urease hydrolysis of urea to ammonia is measured photometricially by Berthelot's reaction to quatify urea.

Urea + H_2O ⟶ $2NH_3 + CO_2$

NH_3+ hypochlorite + phenol ⟶ indophenols (blue compound)

3.2.7.3.2.2 Calculation

Urea concentration = $\frac{A_{sample} \text{ x standard concentration (mmol/1)}}{A_{Standard}}$

Mix and allow to stand at the appropriate temperature for 10 minutes. Then add to a cuvette, and read the absorbance A_1. Read absorbance A_2 exactly 5 minutes later.

3.2.7.3.3Alanine aminotransferase (ALT) and Aspartate Aminotransferase (AST).

ALT and AST are also called serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetate (SGOT) respectively (Evans, 2009). The colorimetric method was used. Exactly 1ml of substrate was pipetted into a glass tube and heated at 37 ^{0}C for 5 minutes using a water bath. Then 0.2 ml of serum was pippeted into the reaction mixture and mixed thouroughly. The mixture was then incubated for 60 minutes for AST and 30 minutes for ALT. Thereafter, 1ml of phenylhydrazine solution was added and mixed. The measurement obtained from the reaction product was subtracted from that of the blank to get the actual value.

3.2.8Qualitative phytochemical analysis

The methanol extract of *Persea americana* root was evaluated for the presence of nine secondary metabolites including alkaloids, anthraquinones, glycosides, flavonoids, phenols, saponins, tannins, triterpines and phytosterols using the procedures described by Prashant *et al*. (2011).

3.2.9 Quantitative Phytochemical Analysis.

3.2.9.1 Total Phenolic Content of *P. americana*Extracts

Total phenolic content (TPC) in the *P.americana* plant extracts was measured by the protocol reported by Wolfe *et al*. (2003). Firstly, 1 mL of the extracts was reacted with 5mL Folin Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 ml (75g/L) of sodium carbonate. The tubes were thouroughly mixed with vortexing machine for 15seconds and incubated for 30 mins at 40^0C to allow reaction to take place evident by a colour change in the reaction mixture. Spectrophotometer (Biochrom WPA Biowave II, Cambridge, England) was then used to measure the absorbance at 765 nm. The calibration curve of gallic acid was used to extrapolate the total phenolic content of the extract, expressed as mg/g gallic acid equivalent.

3.3 OBJECTIVE 3

3.3.1 THE THERAPEUTIC EFFECT OF THE METHANOL LEAF EXTRACT OF PERSEA AMERICANA ON L-NAME INDUCED HYPERTENSION IN WISTAR RATS.

3.3.2 PLANT EXTRACTION

The plant extraction was done as earlier described under objective 2.

3.3.3 EXPERIMENTAL ANIMALS

A hundred and twenty albino rats were purchased for these studies. The rats were randomly selected and acclimatized to the feed and the environment for about three weeks. They were provided with Top pelletized growers rat feed and water ad libitum. Good housing and optimum ventilation were provided. Equal periods (12 hour) of light and darkness were provided during the period of acclimatisation.

3.3.4 EXPERIMENTAL DESIGN

A hundred and twenty male wistar rats were used for this study. They were divided into 12 groups (A, B, C, D, E, F,G, H, I, J, K, L) of ten. A was given distilled water, B was given L-NAME alone, C, D and E were given L-NAME+ PALE (100, 200, 400 mg/kg respectively), F, G and H were given L-NAME+ PABE (100, 200, 400 mg/kg respectively) I, J, and K were given L-NAME+ PARE (100, 200, 400 mg/kg respectively). A daily repetitive dose of L-NAME (at a dosage of 40 mg/kg), lisinopril (at a dosage of 0.28mg/kg) and plant extracts were given for 21days to each rat within the group at designated dose according to the experimental design.

After the study, the blood pressure of each rat was measured without anaesthesia by tail plethysmography using electrosphyngomanometer (CODA, Kent Scientific, U.S.A). An average of five readings were taken and recorded in the quiescent state following acclimatization of the rats. The rats were anesthetized using xylazine and ketamine to ensure proper restraint for electrocardiography. The electrocardiograms of 3-4 rats per group were taken. Blood samples were taken and the rats were sacrificed on day 22 and the heart and kidney were harvested.

3.3.5 Blood Collection and preparation of Serum for Serum Chemistry

After the rats recovered from the anesthesia, blood was collected from each rat via retro-orbital venous plexus bleeding with the aid of clean capillary tubes into dry clean plain tubes and EDTA bottle. The blood collected in the EDTA bottle was gently rocked and was used for hematological studies. The blood collected into clean plain tube was allowed to coagulate and was thereafter centrifuged at 4,000 rpm for 10minutes. Decanted serum was refrigerated at -4^0C. The serum was then used for biochemical analysis.

3.3.6 HAEMATOLOGY

Red Blood cells, white blood cells and platelets parameters were done as described in the previous experiment.

3.3.6.1 SERUM BIOCHEMISTRY

Serum analytes including creatinine, blood urea nitrogen, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured using the protocol described by the manufacturer of the kit used (Randox Ltd, UK)

3.3.6.1.1Serum Creatinine Estimation

Creatinine levels were estimated using the Randox Kitas described in Objective 2 above

3.2.6.1.2 SERUM UREA ESTIMATION

The estimation was carried out using the Randox kit as described in objective 2 above

3.3.6.1.3 Alanine aminotransferase (ALT) and Aspartate Aminotransferase (AST).

The estimation of AST and ALT activities were carried out using the Randox kit as described in Objective 2 above

3.3.7BIOCHEMICAL ASSAY

3.3.7.0. Sample preparation

The heart and kidney were homogenised in phosphate buffer saline and homogenised using mounted electrical homoginizer. The homoginized tissues were centrifuged in cold centrifuge. The supernatant was decounted and kept in freezer until analysed.

3.3.7.1. MALONDIALDEHYDE ASSAY

3.3.7.1.1 REAGENTS AND PROCEDURE FOR MALONDIALDEHYDE

The MDA level was carried out and calculated according to the method described by Adam-Vizi and Seregi (1982). Lipid peroxidation in units/mg protein or gram tissue was computed with a molar extinction coefficient of 1.56×10^5 $M^{-1}Cm^{-1}$.

$$\text{MDA (units/mg protein)} = \frac{\text{Absorbance x volume of mixture}}{E_{532nm} \text{ x volume of sample x mg protein}}$$

3.3.7.2 Catalase Activity

3.3.7.2.1 Procedure for Catalase Activity

Exactly1 ml of dichromate/ Acetic acid solution was measured into all the test tubes. Then a reaction mixture was made in a small beaker placed in ice by adding 2 ml of H_2O_2 solution, 2.5ml of buffer, 0.5ml of properly diluted sample. (Diluted sample was made by adding 0.1ml of sample to 0.4ml of distilled water). The reaction mixture was gently swirled at 26^{o}C. Also 1ml portion of the reaction mixture was added into each of the test tubes containing 1ml dichromate/acetic acid solution, the stop watch was set at 60secs interval, and this was done till the 4th test tube per sample. They were then incubated in the water bath at 100°C for 10 minutes. The reaction products was read at the absorbance at 570nm using distilled water as blank.

3.3.7.2.2 Determination of Catalase Activity

The activity of the enzyme catalase was measured by the procedure described by Claiborne (1985). Determining Catalase Activities (Kinetic)

Precisely 2.95 ml of phosphate buffer containing 19 mM of H_2O_2 was pipette into cuvette and 50 µL of sample was also added. The reaction product was measured using spectrophotometer at 240 nm every 15sand 30s till 3 minutes for the kidney and the heart, respectively.The mixture was mixed by rapid inversion in cuvett before each measurement.

3.3.7.3 Hydrogen Peroxide Generation:

Procedure:

Hydrogen peroxide generation was determined by method describe by Wolff, 1994. Precisely 2500 µL of buffer was measured into all the test tubes, then 250 µL of AFS, a hundred microlitre of sorbitol, a hundred microlitre of Xylenol Orange, 25 µL of sulphuric acid and fiftymicrolitres of of the test samples were added, the mixtures were mixed thoroughly by vortexing till they foamed. They were then left on the bench to incubate at 25^{o}C for 30 minutes. Thereafter, the reaction product was read at 560 nm against water as blank.

3.3.7.4 Superoxide Dismutase Activity

Reagents and Protocol

Reagents:

1. Carbonate buffer (0.05M, pH 10.2)
2. 0.3 mM Adrenaline**:** solutiom of 100 mg of adrenaline in 100 mls of de-ionised water previously mixed with half a millilitre of absolute HCl.

Protocol:

Superoxide dismutase (SOD) enzyme activity was measured by its ability toinhibit the auto-oxidation of epinephrine (pH 10.2) at 30^0C as described (Misra & Fridovich, 1972).

Blank/ control: To prepare blank,20 µL of distilled water and 0.3 ml of Adrenaline solution were measue curvette to which 2.5 ml of carbonate bufferhad been pipetted.

The curvette was quickly inverted, change in absorbance at 480nm was monitored 150 secs at 30 seconds interval.

For the sample: 2.5ml of carbonate buffer was added into the curvette, then 20 µL of sample and 0.3ml of adrenaline solution.The curvette was quickly inverted,and change in absorbance at 480 nm was mornitored for 150secs at 30 seconds intervals.

The formular for determining the percentage inhibition is as follows:

$$\text{Change in readings in 60 seconds} = \frac{A_3 - A_0}{2.5}$$

In which, A_0 = initial reading after seconds

A_3 = Reading at 150 seconds

$$\% \text{ inhibition} = \frac{\text{change in the measurement for substrate}}{\text{change in values of blank}} \times 100$$

3.3.7.5 NITRIC OXIDE ASSAY

Reagents and Preparation of Nitric Oxide Assay

Nitric oxide was measured as described by Olaleye *et al*., 2007

A solution of0.1g of 0.1% N- (1-naphthyl) ethylenediamine dihydrochloride in100ml dH_2O.

Also, 95 ml of distilled water was used to dilute 5ml of phosphoric acid.

Lastly, 1g of Sulfanilic acid was added to mixture of the first and second reagents.

Assay Protocol

In this assay, 50µL of tissue supernatants and 0.1 µL Griess reagent were mixed. Then incubated for 20 minutes at 26 ^{0}C and read at 490 nm with the aid ofmicropipette plate reader. Nitrite concentration was determined by a sodium nitrite ($NaNO_2$) standard curve and was expressed as µmol nitrite/mg protein.

3.3.7.6 MYELOPEROXIDASE ASSAY

Reagents and Procedure for Myeloperoxidase (MPO)

Serum MPO was determined as described by Xia and Zweier, (1997)

O-dianisidine dihhydrochlororide.

Diluted Hydrogen Peroxide (H_2O_2).

4µL of 30% H_2O + 96µL of distilled water.

0.05 M of Potassium Phosphate buffer (pH 6.0)

Procedure

Exactly 70µL of sample (serum) was pipetted into cuvette containing 2mls of O-dianisidine and H_2O_2. The reaction product was read at 0 second, and twice at 30 seconds intervals at 450 nm wavelengths.

Calculation

MPO generated= change in activity/ 1.13*10-2

T1=30-0 and T2=60-30 seconds.

3.3.7.7 PROTEIN THIOL ASSAY

Reagents and Protocol for Protein Thiol as described by Ellman, (1959)

TCA 10% solution in distilled water

Phosphate buffer saline (0.1 M) (pH 7.4)

I dissolved 174.18g of K_2HPO_4 and 136.09g of KH_2PO_4 in 900 ml of dH_2O (pH 7.4) to make PBS and then water was added to make the total volume 1 litre.

Exactly 3.96g of DTNB was dissolved in 0.1 M PBS.

DTNB (Ellman's reagent or 5, 5-dithos-bis-(2-nitrobenzoic acid).) 0.004g/0.1M Phosphate buffer saline.

Procedure

Precisely 250 µL of TCA and 250 µL of sample were added in test tubes. They were then centrifuged at 4,000 rpm for 5 minutes, supernatant was decanted and 20µL of the supernatant was measured into microtitre plate with the micropipette. Then 184µL of Phosphate Buffer Saline and 6µL of DTNB were added. It was read with micro plate reader at wavelength of 405 nm.

3.3.7.8 GLUTATHIONE S-TRANSFERASE ASSAY

3.1.10.8.1 **Reagents and Procedure for Glutathione S-Transferase (GST) Assay**

The protocol described by Habib et al. (1974) was used to determine GST activity

0.1M Potassium Phosphate buffer (pH 6.5)

1-chloro 2, 4-dinitrobenzene (CDNB) was dissolved in 10ml of ethanol.

A 2ml solution of reduced glutathione (GSH) was made in 0.1M phosphate buffer (PH6.5).

Protocol for Glutathione S-Transferase Activity

10µL of sample and 140µL of phosphate buffer saline, 10µL of GSH, and 50µL of CDNB (1-Chloro, 2, 4-dinitrobenzol) were pipetted into micro plate. Half plate was read first then the other half at wavelength of 405nm.

GST unit = µmole/min/mg protein.

3.3.7.9GLUTATHIONE ASSAY

Reagents and Procedure for Glutathione Assay as described by Beutler et al., 1993

Phosphate buffer(pH 7.4) was used to dissolve 40 mg GSH, a total volume of 100ml was prepared.

0.1M Phosphate buffer (pH 7.4).

a) 0.1M K_2HPO_4 .$12H_2O$ (0.992g of salt in 200ml of distilled water).

b) 0.1M KH_2PO_4. $2H_2O$ (MW=156.03) (1.946g of salt in 200ml of distilled water).

Mixture of 200ml of (a) to 100ml of (b) (pH 7.4).was used to prepare 0.1 M phosphate buffer.

3. Ellman's Reagent [5′, 5′-Dithiobis- (2-nitrobenzoate) DTNB]

4. Precipitating Solution

Solution of 4% of sulphosalicyclic acid ($C_7H_6O_6S.2H_2O$ Mol. Wt. 254.22) was prepared.

Procedure

Table for serial dilutions of the GSH working standard.

Table 3.2.1 Preparation of GSH Standard Curve

Stock (ml)	PO4 buffer (ml)	Ellman's Reagent (ml)	Absorbance (412nm)	GSH conc. (µg/ml)
0.02	0.48	4.5	0.04	8
0.05	0.45	4.5	0.101	20
0.10	0.40	4.5	0.194	40
0.20	0.30	4.5	0.380	80
0.30	0.20	4.5	0.572	120
0.40	0.10	4.5	0.749	160

Precisely 4.5ml of Ellman's reagent was measured into each of the mixture.The absorbance of resultant solution was measured at 412nm.

GSH level Determination

Amixture of0.5ml of sample and 0.5ml of the precipitating solution were made in a text tube to react. Then the centrifugation of the mixture was done at 4,000rpm for 5 minutes. A mixture of 0.5 ml of the supernatant and 4.5ml of Ellman's reagent were made in a test tube. The solution was read at 412 nm against blank (distilled water). Extrapolate the GSH using the standard curve above.

3.3.7.10 GLUTATHIONE PEROXIDASE

Glutathione peroxidase activity was measured according to Rotruck*et al.* (1973).

REAGENT	CONCENTRATION
$NaNO_3$	0.0325g in 15mls of distilled water (DW)
GSH	0.123g in 10mls in phosphate buffer
H_2O_2	28uL in 100mls of distilled water (DW)
TCA	2g in 20mls of distilled water (DW)
K_2HPO_4	5.23g in 100mls of distilled water (DW)
DTNB	0.04g solution with phosphate buffer (100mls)
Phosphate buffer	200ml solution of 0.992gof K_2HPO_4 and 1.946g of KH_2PO_4 in DW

PROCEDURE

The reagent into a test tube in the following manner-

100µL of phosphate buffer

20µL of $NaNO_3$

40µL of GSH

20µL of H_2O_2

100µL mls of sample

120µL of distilled water

The mixture was incubated at 37 ^{o}C for 5 minutes, and 100µL of TCA was pipetted into it. This was followed by centrifugation at 4,000revolution per minute for 5minutes. 2mls of K_2PHO_4 and 1ml of DTNB were added to 1ml of supernatant, the resultant mixture was read at 412nm against blank.

Calculation

Consumed GSH/mg protein

= 245.84-remaining GSH

A unit of GPx activity depicts the amount of enzyme required to utilize 1nmol of NADPH/ in onminute at 25^0C.

3.3.7.11 NON PROTEIN THIOL

Reagents for Non-Protein Thiol

Protocol for non-protein thiol (NPSH) as described by Ellman, (1959)

10% TCA in distilled water

0.1 M of phosphate buffer saline (PBS) (pH 7.4)

174.18g of K_2HPO4 136.09g of KH_2PO4 were dissolved in 900 ml of distilled water, (pH 7.4) then top up to1000mlto make PBS.

10 mM DTNB (Ellman's reagent), 3.96 g of DTNB was dissolved in 0.1 M PBS.

Procedure

Exactly 250 µL of TCA and 250 µL of sample were mixed together in a test tube. It was then centrifuged at 4,000 rpm for 10minutes. The supernatant was decanted and 20µL of the supernatant was pipetted into the plate. 170 µL of Phosphate Buffer Saline and 10 µL of DTNB were reacted with it. .The resultant mixture was read with micropipette plate reader at wavelength of 405 nm.

3.3.7.12 DETERMINATION OF PROTEIN CARBONYL

Protein carbonyl was determined by the protocol described by Lowry et al., 1951

Principle

DNPH + protein carbonyl –2,4-dinitrophenylhydrazone which can be read with a spectometeReagents

1. 10 Mm DNPH in 2M HCl

A solution of 0.099 g of DNPH in 50 ml of 2M HCl was prepared. (made by taking 9.8 ml of concentrated HCl into 40.18 ml of distilled water).

2. 20% TCA-

Dissolve 20g of TCA in 100 ml of distilled water.

3. Absolute Ethanol

4. 6 M Guanidine HCl (Dissolve 573 g of Guanidine HCl in 1 litre of distilled water).

Absolute ethyl Acetate

Procedure

Amixture of 500 µL of DNPH solution and 100 µL of sample was made in a test tube and the mixture left on the bench to incubate for incubated for one hour at 26 ^{o}C. It was then mixed by vortexing the sample every 15 minutes. This was followed by addition of 500 µL of TCA and the test tubes were placed in ice for 5 minutes. Then, the protein precipitate was collected by were then centrifuging the mixture at 4,000 rpm for 10 minutes. The washing of the precipitate was done two times with 1ml of ethanol-ethyl acetate (v/v). Then, 600 µL of Guanidine HCl solution was used to dissolve the final precipitate. The reaction mixture put in incubator for 15 minutes at 37℃ and read spectcometrically at 370 nm using distilled water as blank.

Calculation**:**

.Thecarbonyl contentwas calculatedbased on themolarextinction coefficientofDNPH(e¼2.2 10^4 cm^1 M^1) innmoles/mgprotein.

3.3.8 IMMUNOHISTOCHEMISTRY

3.3.8.1Immunohistochemistry of the NFkB in the heart and kidney

The tissues of the heart and kidney harvested and stored in buffered formalin were embedded in paraffin, sectioned and processed for immunohistochemistry on charged slides. Melting of the paraffin was done using oven at 60^0C. Xylene was then used to dewax. The dewaxed tissues were passed through ethanol of decreasing concentration (100%-80%). The tissue were also bath in H_2O_2/ methanol for peroxidase quenching. The antigen retrival was done by heating the tissue in 0.01M citrate buffer (PH 6.0) with the aid of a microwave. Normal goat serum (10%, HistoMark®, KPL, Gaithersburg, MD, USA) was used to block all the sections and then probed with ERK, HSP70 and cytochrome C antibodies (Abclonal). The antibody detection and treatment with Horseradish peroxidase streptavidin were done as described by the manufacturer of the kit (HistoMark® , KPL, Gaithersburg MD,USA).The rection product was stained diaminobenzidine (DAB, Amresco, USA) for 6-10 minutes, later counterstained with high definition hematoxylin (Enzo®, NY_USA). The resulting product was dehydrated in increasing concentration of ethanol. Then mountant was added to the section on the slides and covered with coverslips. Slides were then viewed with 400 × magnifications with a photo microscope (Olympus) and a digital camera (Tuopcam, Touptek Photonics, Zhejiang, China). Immunohystochemistry was described as reported byOyagbemi *et al.* (2017).

3.4 OBJECTIVE 4

3.4.0 PHYTOCHEMISTRY OF PERSEA AMERICANA ROOT AND ISOLATION OF PURE COMPOUNDS FROM THE ROOT AND LEAVE EXTRACTS

3.4.1 COMPOUND ISOLATION TECNIQUES

3.4.1.1 COLUMN CHROMATOGRAPHY

The Column chromatography is a simple cost effective method commonly used in chemistry to separate and purify chemical compounds from a mixture of compounds. Column chromatography separate compounds based on polarity and retention time if silica gel is used as stationary phase, and it separates based on molecular weight if cephalex is used as the solid phase.

3.4.1.2 Silica Gel Column Chromatography

The column was blocked at the outlet with a ball of cotton wool and the column was loaded with pure dry silica (50 g) as the stationary phase and 20 g of extract was mixed with another portion of dry silica. The dry mixture of silica and extract was then loaded on the pure silica in the column. The solvent phase was derived from a non-polar solvent (N-hexane) to a medium polar (Ethyl acetate) to a polar solvent (Methanol) The solvent phase used was derived from the combination of solvent originally developed that separated the compound on thin layer column.

The solvent combination was: Ethylacetate (2): Acetone (2): Methanol (0.5): Water (0.5).

The column chromatography procedure is shown in figure 3.4.1 as was run from a non-polar (N-Hexane) to the polar (Methanol). The separated or eluted compounds were collected into glass container through the top of the column. Average of 50ml of the compounds were collected serially into glass containers until the compounds were fully separated.

Figure 3.4.1 Column chromatography using silica

Metabolytes are collected into bottled small volumes of 50 ml in glass containers as the silica and extract mixture are washed through the column using different solvent from non-polar solvents such as N-Hexane to polar solvent like Methanol

The collected compounds were then tested with T.L.C. plates to check purity and to determine the retension/ retarding factor (RF) so that compounds with similar RF were pooled together (as shown in Tables 3.3.1 and 3.3.2).

Figure 3.4.2: Separated plant metabolytes by polarity into glass containers. A total of 356 glass containers were used.

Table 3.3.1 Pooled Subfractions of the Ethyl Acetate Fraction of the *P. americana* leaf

A	-	1 - 40	A
B	-	41 – 60	B
C	-	61 – 79	C
D	-	80 – 99	D
E	-	100 - 104	E
F	-	105 – 119	
G	-	120 – 140	F
H	-	141 – 145	G
I	-	146 – 154	H
J	-	155 – 170	I
K	-	171 – 206	J
L	-	207 – 211	
M	-	212 – 246	K
N	-	247 – 251	L
O	-	252 – 256	M
P	-	257 – 261	N
Q	-	262 – 268	
R	-	269 O	
S	-	270 – 279	
T	-	280 – 302	P
U	-	303 – 312	Q
V	-	313 – 356	R

The 356 filtrates/ metabolites were separated using TLC. Similar compounds under UV lamp were pulled together and labelled A-V on first examination and later to A-R when the process was repeated.

Figure 3.4.3: Thin layer chromatography used to merge similar metabolites as viewed under UV light.

Table 3.3.2 Pooled Subfractions of the Ethyl Acetate Fraction of the *P. americana* leaf and the yield after the metabolite was oven dried.

	2nd pooling	**Net yield (g)**
1	A – 1 - 40	0.4363
2	B – 41 - 60	0.1971
3	C – 61 – 79	0.412
4	D - 80 – 99	0.2484
5	E – 100 – 119	2.3384
6	F - 120 – 140	1.0408
7	G - 141 – 145	0.8172
8	H – 146 – 154	1.1614
9	I - 155 – 170	3.7043
10	J – 171 – 214	14.4388
11	K – 212 – 246	2.2512
12	L – 247 – 251	0.3642
13	M – 252 – 256	0.4321
14	N – 257 – 261	0.492
15	O – 262 – 279	0.7095
16	P – 280 – 302	0.6927
17	Q – 303 – 312	0.4904
18	R – 313 - 353	1.2981

Figure 3.4.4: UV lamp for viewing UV light sensitive compounds on thin layer chromatography plate

3.4.1.3 Cephalex Column Chromatography

This form of chromatography separate compounds by molecular weight. The long column was blocked with a cotton bud and the cephalex solid phase was mixed with methanol and poured into the column. It was then allowed to settle with the column tap locked. When it was observed to have settled, the methanol was allowed to flow and was collected in test tube.

The fraction of extract was then loaded when the methanol was very low on the cephalex solid phase. The subfraction was fully loaded and was left to be absorbed in the solid phase. Methanol was used as the solvent phase for the cephalex column chromatography and 10ml of the eluted compounds were collected into the test tubes to further separate the subfraction into individual compounds.

Figure 3.4.5: Cephalex column during metabolite isolation process.

3.4.1.4 Thin Layer chromatography

This is a chromatography in which compounds are separated on a thin layer of absorbent materials typically a coating of silica gel on a glass, or aluminium plate or rubber sheet.

A line (spotting line) was drawn with pencil parallel to and 1cm from the bottle line of the silica coated aluminium TLC plate. About 0.2 ml of each fraction was spotted on the TLC Plates using a capillary tube filled with the fraction. The spotted plate was then allowed to dry. This was then put in solvent combination or system predetermined and found to be capable of separating the sub-fractions based on polarity of the extract fractions. The plate was allowed to develop as the solvent moved up the TLC plate to the maximum level (solvent front). The TLC plates were removed from the solvent tank, left on the bench to dry and the solvent front was marked with pencil. And the separated compounds were visualized on 250 – 354 nm ultraviolent lamp and the UV light sensitive compounds with shower as bright fluorescent and were marked by drawing a ring around them with pencil (Figure 3.4.5).

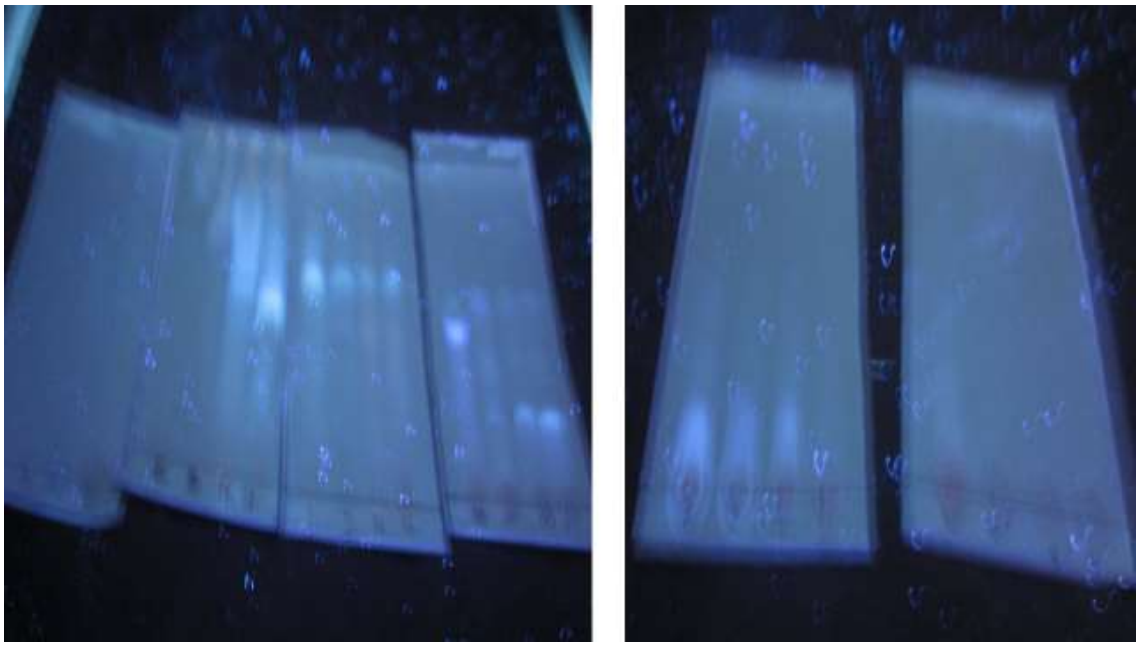


Figure 3.4.6: Thin layer chromatography and metabolytes isolation from the *P.americana* extract used in pooling of metabolytes with similar Rf.

With this procedure, fractions with similar retention factor (Rf) were pooled together. The TLC analysis carried out at room temperature in the laboratory. Some solvent system used for the thin layer chromatography were as listed below:

Solvent system: N- Hexane 1.5: Ethyl acetate 3.5

Ethylacetate: Toluene: Methanol: 3:1:1:1

Chloroform: Ethanol: Water 2.5:1.5: 0.5

Ethanol: Toluene: Water 4: 1.5: 0.5

Retention factor (RF)calculation:

RF = distance moved by solute

Distance moved by solvent from the origin to the solvent front

The metabolites having the same RF were pooled together and dried in rotary evaporator for further purification using preparatory thin layer chromatography.

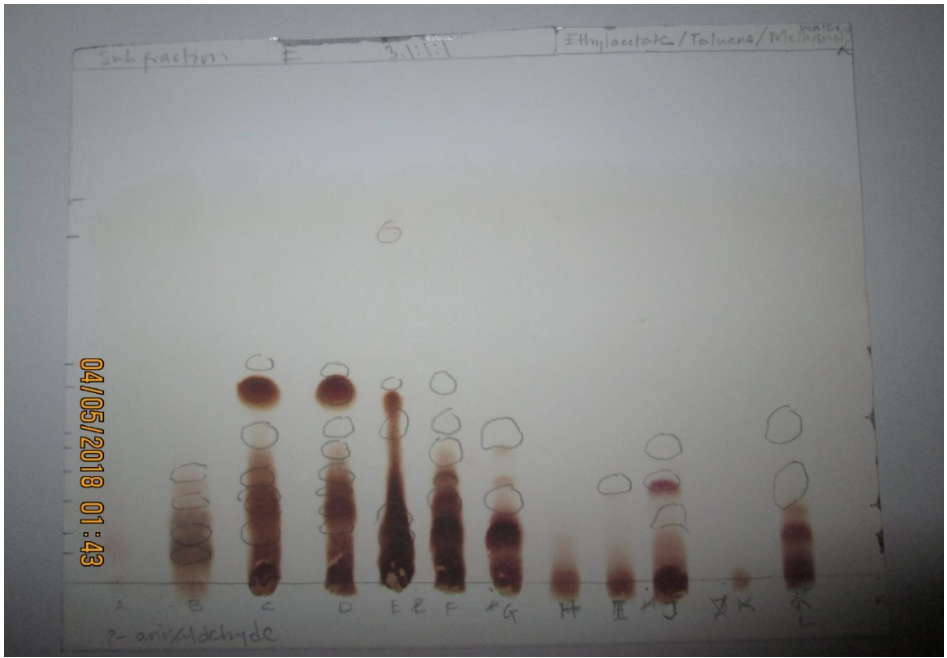


Figure 3.4.7: The separated metabolytes in the subfractions of the ethyl acetate fraction of the *P.americana* root on TLC plates viewed underUV lamp before and after staining with P-anisaldehyde. Subfractions A-S.

The compounds that were not UV-light sensitive were identified by counter stain on the TLC plate with P-anisaldehide staining agents which colour different compounds differently and the stained TLC plates were also viewed under UV lamp with special protective viewing glass.

Figure 3.4.8The process of phytocompound isolation using UV lamp

3.4.1.5 Preparative Thin Layer Chromatography (Prep- TLC)

Preparative TLC was used for analytical separation of large quantity of materials or complex compounds into simple units. It was also used for chemical samples. It uses the same principle of thin layer chromatography. The plates are available commercially as glass, aluminium foil or plastic plates coated with silica gel. The plates can also be prepared in the laboratory by coating the glass with an adsorbent material such as silica gel, aluminium oxide or cellulose (Stationary phase). The absorbent was mixed with small amount of binder like calcium sulphate (gypsum) and water. The mixture was spread as thick (0.5-2mm) coat on the glass plates. The mobile phase comprised of solvent or solvent mixture that could separate complex compound to constituent simple compounds. The solvents move up the plate through capillary action.

The solvent or solvent combination were poured into a tank and allowed to saturate the tank before the spotted plate was placed. The solvent was allowed to run up the plate but not allowed to overrun the plate. The point where the movement was stopped is called solvent font. The separated compounds were viewed under UV lamp and mapped out with a pencil. The mapped out compound was teased out with a razor blade into separate beakers. Separated compounds were recovered from the absorbent by mixing the teased out absorbent with methanol. The methanol and dissolved compounds were filtered out and the filterate concentrated to recover respective compounds.

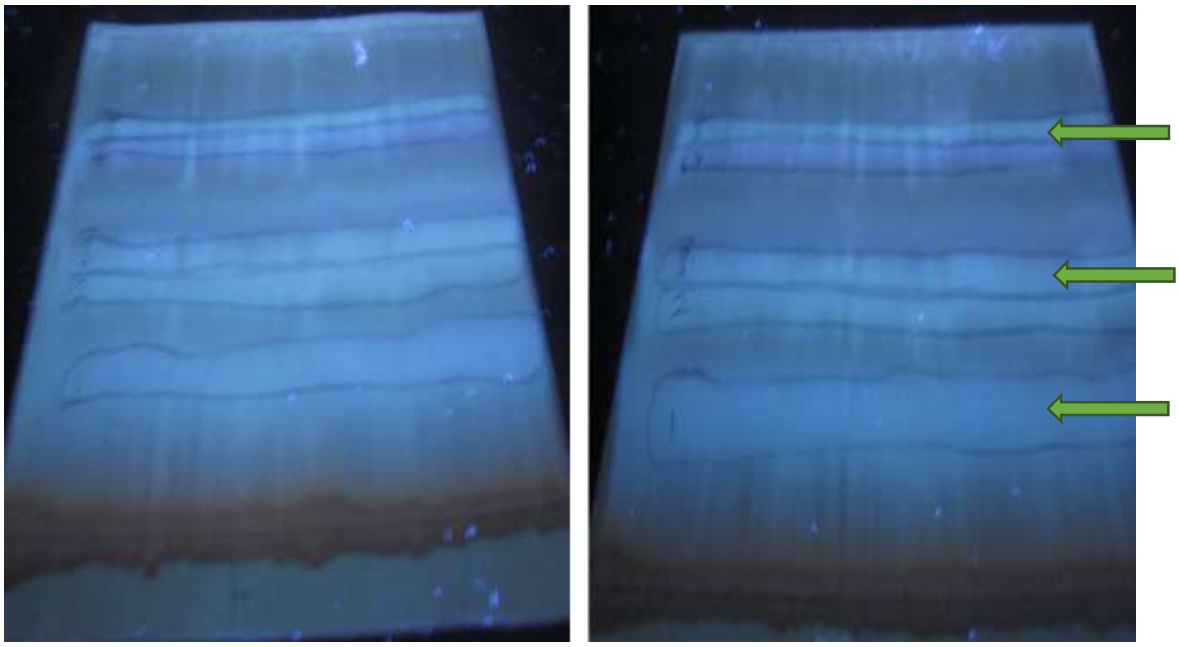


Figure 3.4.9: Preparative thin layer chromatography and metabolytes separation from the *P.americana* leaf and root extracts. (Viewed with UV lamp). Seperated compounds traced out with pencil as shown by UV radiation (green arrows).

3.4.2 Determination of Bioactive Metabolites

In the isolation process, there is need for the isolation of active metabolites that are directly involved in the activities found in the crude extracts. The activities of the ethyl acetate subfractions were evaluated by DPPH radicals scavenging activities as described by Torkoglu *et al*. (2007) and the total phenolic content as described by Wolfe *et al*. (2003). Details is as described above. Subfractions E, F and O had the higher antioxidant activities compared to other subfractions and were processed further for phytocompound isolation

3.4.2.1 Isolation Process from the Leaf Extractby Thin Layer Chromatography

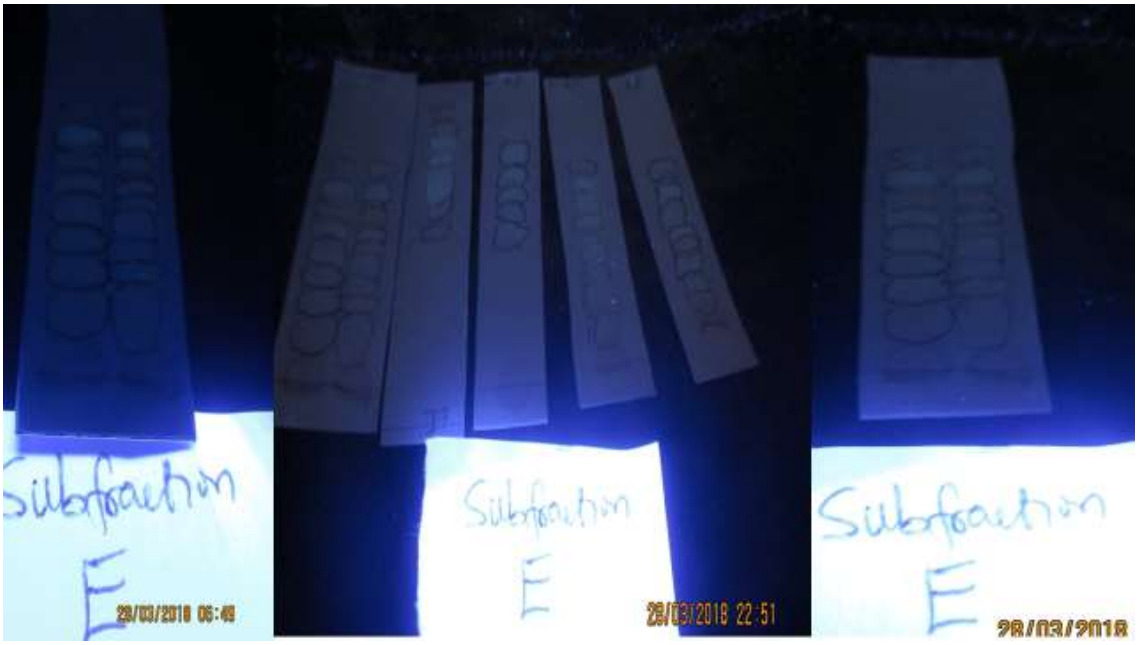
The ethyl acetate subfraction E of the leaf was separated into single phytocompounds using thin layer chromatography. In this process, different solvent system were used until I arrived at the one that gave the best separation of the constituent compounds. The TLC were air dried and then viewed under UV light in a dark room each time a solvent system was tried(Figure 3.4.10).

Approved Solvent system: Ethyl acetate: Toluene: Methanol: Water 3:1:1:1

Figure 3.4.10: Thin layer chromatography and metabolytes separation from the *P.americana* leaf extract.

3.4.2.2 Isolation Process from the Leaf Extractby Thin Layer Chromatography

The ethyl acetate subfraction E of the leaf was separated into single phytocompounds using thin layer chromatography. The solvent system used was chloroform, ethanol and water mixed in coupling jar, airied and viewed under UV light in a dark room (Figure 3.4.11).



Approved solvent system: Chloroform: Ethanol: Water 2.5: 1.5: 0.5

Figure 3.4.11: Thin layer chromatography and metabolytes separation from the *P.americana* leaf extract.

3.4.2.3 Isolation Process from the Leaf Extractby Thin Layer Chromatography

The separation process of subfraction E of the ethylacetate subfractionof the leaf into constituent compounds was done. The plates were viewed with the aid of UV lamp but but for the sake of compounds that are not UV-light sensitive I counterstained the TLC plate with P-anisaldehyde staining agents which colour different compounds differently. The stained TLC plates were also viewed under UV lamp with special protective viewing glass (Figure 3.4.12)

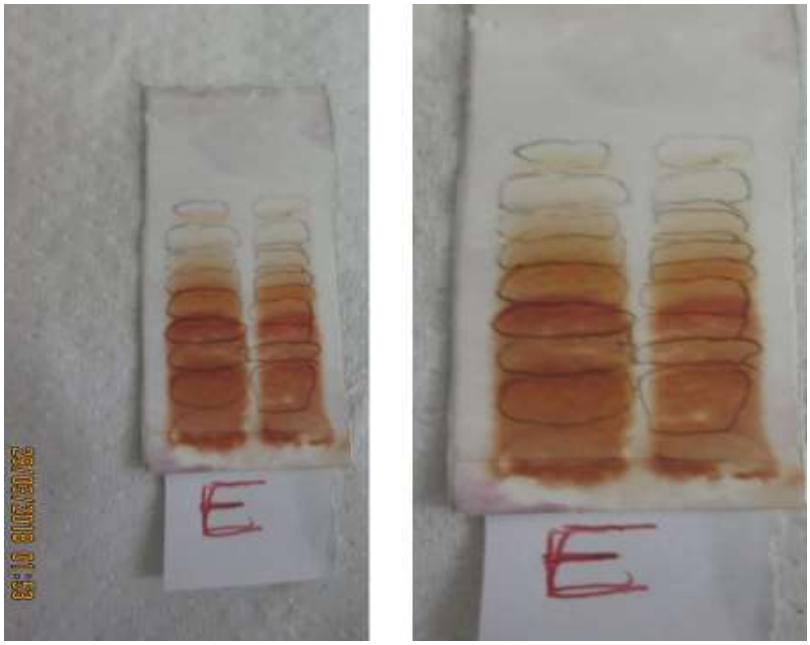


Figure 3.4.12: Thin layer chromatography and metabolytes separation from the *P.americana* leaf extract stained with a staining agent (P- anisaldehyde). (Viewed with UV lamp)

3.4.2.4 Isolation Process from the Leaf and Root Extractsby Thin Layer Chromatography

The ethyl acetate subfractions F of the leaf and root were separated into single phytocompounds using thin layer chromatography. In this process, different solvent system were used until I arrived at the one that gave the best separation of the constituent compounds. The TLC were air dried and then viewed under UV light in a dark room each time a solvent system was tried (Figure 3.4.13).

Figure 3.4.13: Thin layer chromatography and metabolytes separation from the *P.americana* leaf and root extracts. (Viewed with UV lamp)

3.4.2.5 Isolation Process from the Leaf and Root Extractsby Thin Layer Chromatography

The separation process of subfraction F, E and O of the ethylacetate subfraction of the leaf and root into constituent compounds was done. The plates were view with the aid of UV lamp but but for the sake of compounds that are not UV-light sensitive I counterstained the TLC plate with P-anisaldehyde staining agents which colour different compounds differently. The stained TLC plates were also viewed under UV lamp with special protective viewing glass (Figure 3.4.14)

Figure 3.4.14: Thin layer chromatography and metabolytes separation from the *P.americana* leaf and root extracts. (Stained with p-anisaldehyde and viewed with UV lamp)

3.4.2.6 Isolation Process from the Root Extractby Thin Layer Chromatography

The ethyl acetate subfraction O of the root extract was separated into single phytocompounds using thin layer chromatography. In this process, different solvent system were used until I arrived at the one that gave the best separation of the constituent compounds. The TLC were air dried and then viewed under UV light in a dark room each time a solvent system was tried (3.4.15).

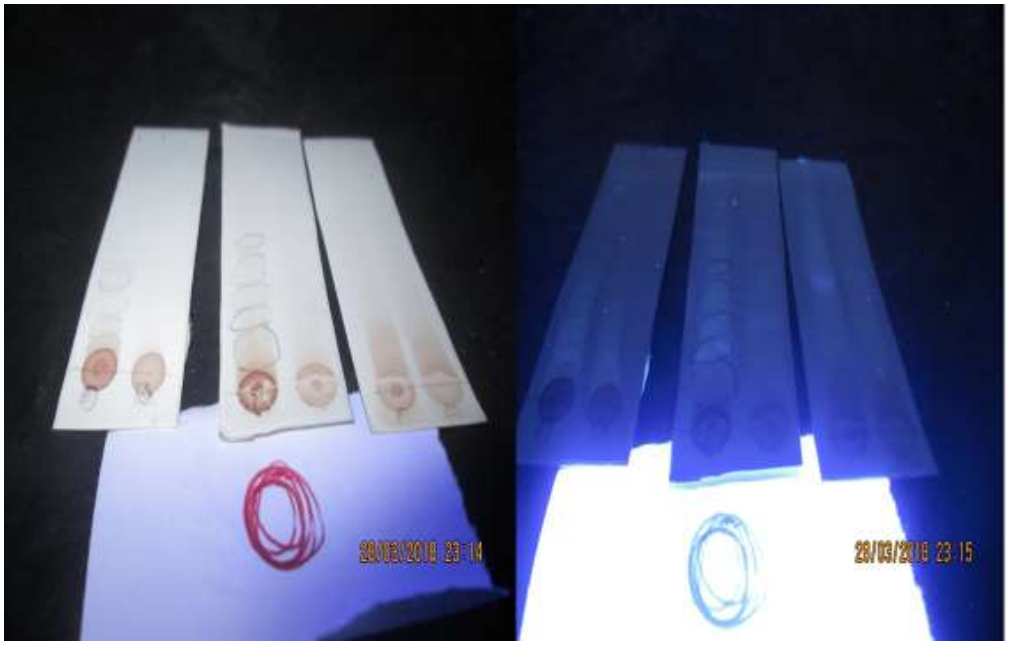


Figure 3.4.15: Thin layer chromatography and metabolytes separation from the *P.americana* root extract. (Viewed with UV lamp)

Approved solvent system was Ethanol: Toluene: Water: 4:1.5:0.5

3.4.2.7 Isolation Process from the Leaf and Root Extractsby Preparative Thin Layer Chromatography

The solvent system for each subfraction was the used in a bigger glass tank with a cover for the separation of the compound using Prep-TLC plates. The plates are allowed to separate while the process is being monitored and the plate removed when the solvent travelled to the line drawn and marked as the solvent font. The plate was air dried using a drier and then view under UV lamp to check the separated compounds. (Figure 3.4.16). The separated compound were marke with pencil, scooped out separately, dissolved in methanol, filtered to separate the plate gel from the compounds and then concentrated to recover the compound

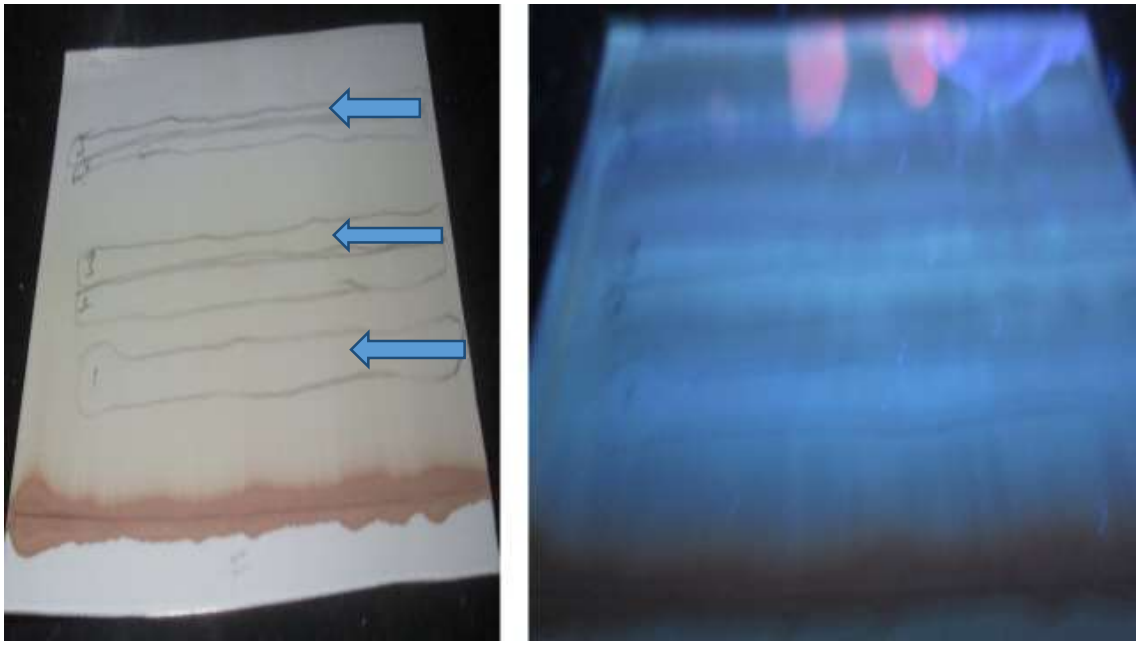


Figure3.4.16: Preparatory thin layer chromatography (Prep-TLC) and metabolytes separation from the *P.americana* leaf and root extracts. (Viewed with UV lamp). The pencil tracings are showing the separated compounds (Blue arrows)

3.4.2.8 Isolation Process from the Leaf and Root Extractsby Thin Layer Chromatography

Purity of the separated compound was done by TLC separation technique using the approved solvent system. Appearance of only one compound on the TLC plate when view under UV light, with and without acid staining confirmed purity (Figure 3.4.17)

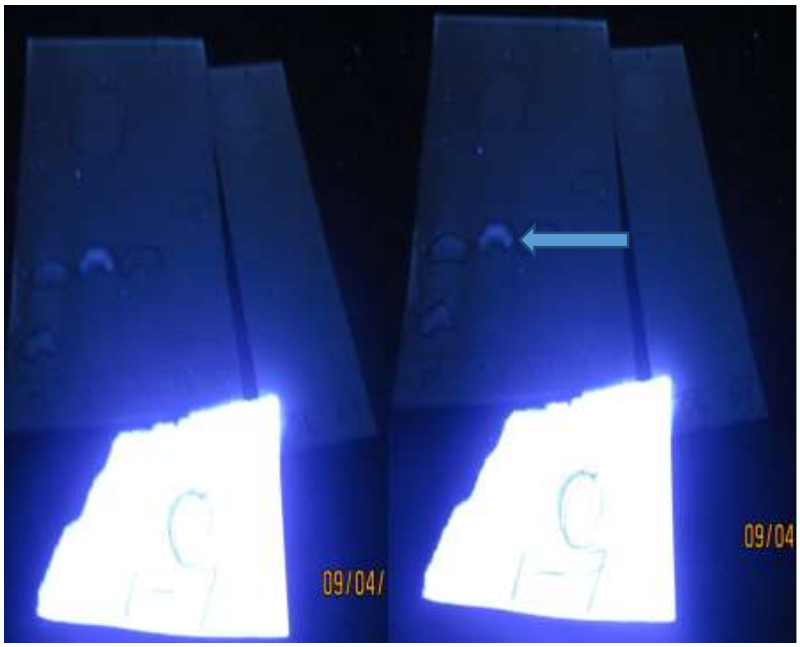


Figure 3.4.17: Preparatory thin layer chromatography and metabolytes separation from the *P.americana*ethylacetate root subfraction O. (Viewed with UV lamp)

3.4.3 Nuclear Magnetic Resonance (NMR) spectroscopy

NMR Spectroscopy is based on pulse variant and it is used for structural elucidation of pure compounds: The groups of pulses may be purely radio frequency (RF) or include magnetic gradient pulses. FT NMR technique, a pulse irradiation technique, was used at ambient temperature.

Two dimension NMR (2D-NMR) technique were used because it saves time and it highlights the two connectivity between different types of nuclei (e.g proton and carbon).

Proton (^{1}H) and carbon –13 (^{13}C) operating at 200MHz for proton and 50MHz for carbon nucleaurNMR spectra were recorded on Bruker Avace^{R} Software. The position of proton and carbon resonance in the NMR spectrum were measured relative to it resonance position of tetraethylsilane (TSM) as internal standard. Deuterated solvents were used to measure NMR spectra. The chemical shifts were indicated in part per million (PPM) with the solvent shift as reference and coupling constrains J. in Hertz (Hz). The pure fractions were dissolved in these solvents after which it was poured inside the NMR tube and the spectra of the compound obtained.

3.4.4FOURIER-TRANSFORM INFRARED (FTIR) SPECTROSCOPY

Fourier-transform infrared (FTIR) spectroscopy is a technique used to obtain an infrared spectrum of absorption or emission of a solid, liquid or gas.

An FTIR Spectroscopy is a technique that helps in analysing chemical compounds like organic, polymeric and inorganic materials. It uses infrared light to view test samples and observe chemical properties. It offers qualitative or quantitative analysis for organic and inorganic samples. It produces infrared spectrum to enable us identify chemical bonds in a molecule. The isolated compounds was analysed using FTIR spectroscopy.

3.5 OBJECTIVE 5

3.5.0 INVITRO ANTIOXIDANT, ANTI- INFLAMMATORY AND ANTI-HYPERTENSIVE ASSAYS OF THE ISOLATED PURE COMPOUNDS.

3.5.1*IN VITRO* ANTIOXIDANT ASSAYS

3.5.1.1 The 1, 1- Diphenly – 2 Picrylhydrazyl (DPPH) Radical Scavenging Assay

The antioxidant activities of the isolated*P. americana*compounds were determined by measuring their ability to decolourize the purple- coloured methanol solution of DPPH, as described by Turkoglu *et al.* (2007).

3.5.1.2Nitric Oxide Scavenging Ability

The capacity of *P.americana* extracts to scavenge nitric oxide radical was evaluated using the procedure reported by Garrat (1964).

3.5.1.3 Metal Chelating Ability of the Extracts

The metal chelation ability of *P. americana* compounds was assayed following the procedure of Dinis *et al*. (1994).

3.5.1.4 Reducing Power Assay

The reduction power activity of the pure compounds was assessed according to the method of Oyaizu (1986).

3.5.1.5 The2, 2’ Azinobis (3-Ethylbenzothiazoline- 6-Sulfonic Acid) ABTS Radical Scavenging Ability

The test was conducted using the procedure of Re *et al*. (1999).

3.5.1.6 Hydroxyl Radical Inhibitory Potential

The ability of the various pure compounds to stop Fe_2^+/ H_2O_2induced decomposition of deoxyribose was assayed using the modified method of Oboh *et al*. (2007).

3.5.2. Angiotensin I converting enzyme (ACE) inhibition assay

ACE inhibition assay was performed followingthe protocol described byOboh *et al*.(2012) and Lin and Li, (2012).

3.5.3 In-vitro Anti-inflammatory Assay.

The 15-LOX (Sigma) anti-inflammatory assay was carried out on the isolated compound as described by Adebayo et al. (2015).

IC50 was calculated using the IC 50 calibration curve.

3.6 Analysis of Data.

The data were analysed and presented as mean ±Standard deviation, the tests of significance were carried out using Student t-test and ANOVA as appropriate. Turkey post-test was also performed using GraphPad Prism version 5.0. The statistical significance was at $\alpha_{0.05}$

CHAPTER FOUR

Results

4.1 Results of the *In vitro* Antioxidant, Anti-inflammatory and Anti-hypertensive Assays of the Crude*P. americana*Extract and Fractions

Table 4.1.1: The *invitro* biological assay results of methanol leaf extract and fractions of *Peasea americana* and gallic acid

EXTRACT/ FRACTIONS	MEAN±STANDARD ERROR OF MEAN ABTS ASSAY]	DPPH ASSAY	HYDROXYL ASSAY	METAL CHELATING ASSAY	N. OXIDE ASSAY
A:PLCM	104.03±11.93	110.63±0.49	108.76±14.39	67.24±14.18	105.64±8.32
B:PLNH	59.66±13.08	97.65±0.17	420.77 ± 43.97^{a}	148.11±33.38	64.26±18.77
C:PLCF	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:PLEA	70.61±13.79	95.02 ± 0.12^{c}	119.37 ± 17.98^{bc}	121.13 ± 6.02^{c}	114.14±12.98
E: PLNB	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:PLAM	165.92 ± 14.99^{abde}	212.11 ± 4.51^{abcde}	25.40 ± 7.88^{abcde}	137.89 ± 4.64^{c}	332.01 ± 33.72^{abde}
G: GALLIC ACID	946.64 ± 29.93^{e}	113.57 ± 6.64^{cef}	58.71 ± 1.94^{bce}	184.09 ± 12.73^{a}	220.29 ± 12.38^{abde}

Group A: *P. americana* leaf crude methanol extract (PLCM), Group B: *P.americana* leaf N-Hexane fraction (PLNH), Group C: *P.americana* leaf Chloroform fraction (PLCF), Group D: *P.americana* leaf Ethylacetate fraction (PLEA), Group E: *P.americana* leaf N-Butanol fraction (PLNB), Group F: *P.americana* leaf Aqueuos methanol fraction (PLAM), Group G: Gallic acid (GA).Significance at $\alpha_{0.05}$, Means with superscript a-g.showed significance when compared with groups A-G. respectively.

Table 4.1.2: The mean and standard deviation of OD values of *P. americana* leaf extract and fractions and gallic acid in reducing power assay.

CONC.	A: PLCM	B: PLNH	C: PLCF	D: PLEA	E: PLNB	F: PLAM	G: GA
15.63	0.029±0.016	0.29±0.032	0.28±0.009	0.38±0.034	0.42±0.006	0.33±0.008	0.46±0.046
31.25	0.32±0.023	0.28±0.014	0.297±0.027	0.46±0.0368	0.65±0.104	0.396±0.012	0.504±0.083
62.50	0.36±0.025	0.32±0.012	0.41±0.73	0.64±0.07	0.74±0.005	0.48±0.018	0.746±0.037
125	0.52±0.035	0.45±0.019	0.51±0.022	0.96±0.021	0.95±0.005	0.64±0.009	$1.048±0.019^{b}$
250	0.54±0.26	0.70±0.030	0.67±0.004	0.96±0.009	0.93±0.005	0.85±0.012	$1.3250±0.059^{abc}$

Group A: *P. americana* leaf crude methanol extract (PLCM) Group B: *P.americana* leaf N-Hexane fraction (PLNH), Group C: *P.americana* leaf Chloroform fraction (PLCF), Group D: *P.americana* leaf Ethylacetate fraction (PLEA), Group E: *P.americana* leaf N-Butanol fraction (PLNB), Group F: *P.americana* leaf Aqueuos methanol fraction (PLAM), Group G : Gallic acid (GA). Significance at $\alpha_{0.05.}$ Means with superscript a - g.showed significance when compared with groups A - G. respectively.

Table 4.1.3: The IC 50 values of *P. americana* leaf extract and fractions, Quercetin and indomethacin in anti-inflammatory assay.

Extracts, fractions& standards	Mean ± SEM
A: PLCM	191.53 ±15.2
B: PLNH	100.24±7.63[a]
C: PLCF	106.42±0.81[a]
D: PLEA	100.86±2.77[a]
E: PLNB	75.36±4.03[a]
F: PLAM	96.65±8.12[a]
G: Quercetin	59.33±5.4[abcdf]
H: Indomethacine	115.09±1.62[aeg]

Group A: *P. americana* leaf crude methanol extract (PLCM) Group B: *P.americana* leaf N-Hexane fraction (PLNH), Group C: *P.americana* leaf Chloroform fraction (PLCF), Group D: *P.americana* leaf Ethylacetate fraction (PLEA), Group E: *P.americana* leaf N-Butanol fraction (PLNB), Group F: *P.americana* leaf Aqueuos methanol fraction (PLAM), Group G: Quercetin, Group H: Indomethacin. Significance at $\alpha_{0.05.}$ Means with superscript a - h.showed significance when compared with groups A - H respectively.

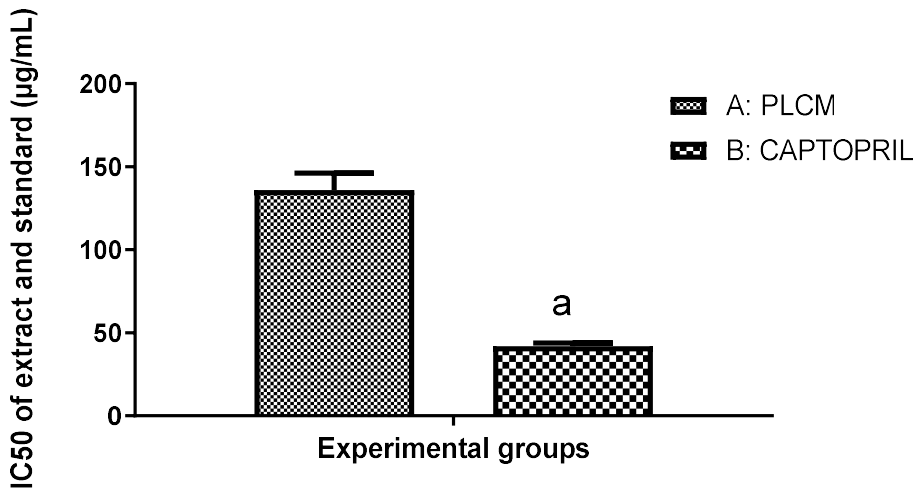


Figure 4.1.1: The IC 50 values of *P. americana* leaf extract in anti-hypertensive assay

Group A: *P. americana* leaf crude methanol extract (PLCM). Group B: Captopril. Significance at $\alpha_{0.05.}$ The anti-hypertensive activity of captopril was significantly higher ($\alpha_{0.05}$) than that of the PLCM.

Table 4.1.4: The IC 50 values of *P. americana* bark extract and fractions and gallic acid in Invitro Biological assay

EXTRACT/ FRACTIONS	MEAN±STANDARD ERROR OF MEAN				
	ABTS ASSAY	DPPH ASSAY	HYDROXYL ASSAY	METAL CHELATING ASSAY	N. OXIDE ASSAY
A:PBCM	28.50±3.65	102.73±0.65	334.19±140.17	204.93±2.20	402.76±25.31
B:PBNH	103.91±5.38	414.76±4.10^{a}	475.36±29.61	267.68±8.17	190.07±10.23a
C:PBCF	166.56±31.68^{a}	91.87±0.0.87^{b}	275.55±20.37	291.72±29.11	886.69±66.20^{ab}
D:PBEA	110.65±33.68	114.96±0.46^{b}	275.18±442.96^{abcfg}	238.48±9.89	266.55±15.50^{c}
E:PBNB	139.92±7.29^{a}	679.61±5.52^{abcd}	209.07±185.72^{abcfg}	136.65±27.07^{bcd}	163.89±2.08^{ac}
F:PBAM	220.33±24.78^{abd}	619.73±23.65^{abcde}	7.08±3.04^{de}	42.48±7.74^{abcde}	297.09±55.88^{c}
G:GALLIC ACID	26.60±4.62^{cef}	113.57±6.64^{bef}	58.71±1.94^{bde}	245.61±50.03^{ef}	89.51±24.91^{cdf}

Group A: *P. americana* bark crude methanol extract (PBCM) Group B: *P.americana* bark N-Hexane fraction (PBNH), Group C: *P.americana* bark Chloroform fraction (PBCF), Group D: *P.americana* bark Ethylacetate fraction (PBEA), Group E: *P.americana* bark N-Butanol fraction (PBNB), Group F :*P.americana* bark Aqueuos methanol fraction (PBAM), Group G : Gallic acid (GA). Significance at $\alpha_{0.05}$

Table 4.1.5: The Means and Standard deviation of the OD values of *P. americana* bark extract and fractions and gallic acid in reducing power assay

CONC.	*A: PBCM*	*B: PBNH*	*C: PBCF*	*D: PBEA*	*E: PBNB*	*F: PBAM*	*G:GA*
15.63	*0.332±0.021*	*0.277±0.017*	*0.399±0.038*	*0.44±0.044*	*0.442±0.044*	*0.291±0.03*	*0.46±0.046*
31.25	*0.408±0.034*	*0.283±0.014*	*0.408±0.062*	*0.55±0.042*	*0.55±0.042*	*0.321±0.03*	*0.504±0.083*
62.50	*0.49±0.065*	*0.329±0.028*	*0.532±0.085*	*0.802±0.025a*	*0.43±0.025*	*0.396±0.02*	*0.746±0.037a*
125	*0.814±0.081*	*0.427±0.011*	*0.762±0.072*	*0.834±0.019a*	*0.834±0.019*	*0.48±0.031a*	*1.048±0.019a*
250	*0.858±0.029*	*0.61±0.024*	*1.048±0.135a*	*0.992±0.053a*	*0.99±0.053*	*0.699±0.07*	*1.3250±0.06a*

Group A: *P. Americana* bark crude methanol extract (PBCM) Group B: *P.americana* bark N-Hexane fraction (PBNH), Group C: *P.americana* bark Chloroform fraction (PBCF), Group D: *P.americana* bark Ethylacetate fraction (PBEA), Group E :*P.americana* bark N-Butanol fraction (PBNB), Group F : *P.americana* bark Aqueuos methanol fraction (PBAM), Group G : Gallic acid (GA). Significance at $\alpha_{0.05.}$Means with superscript a - g.showed significance when compared with groups A - G. respectively.

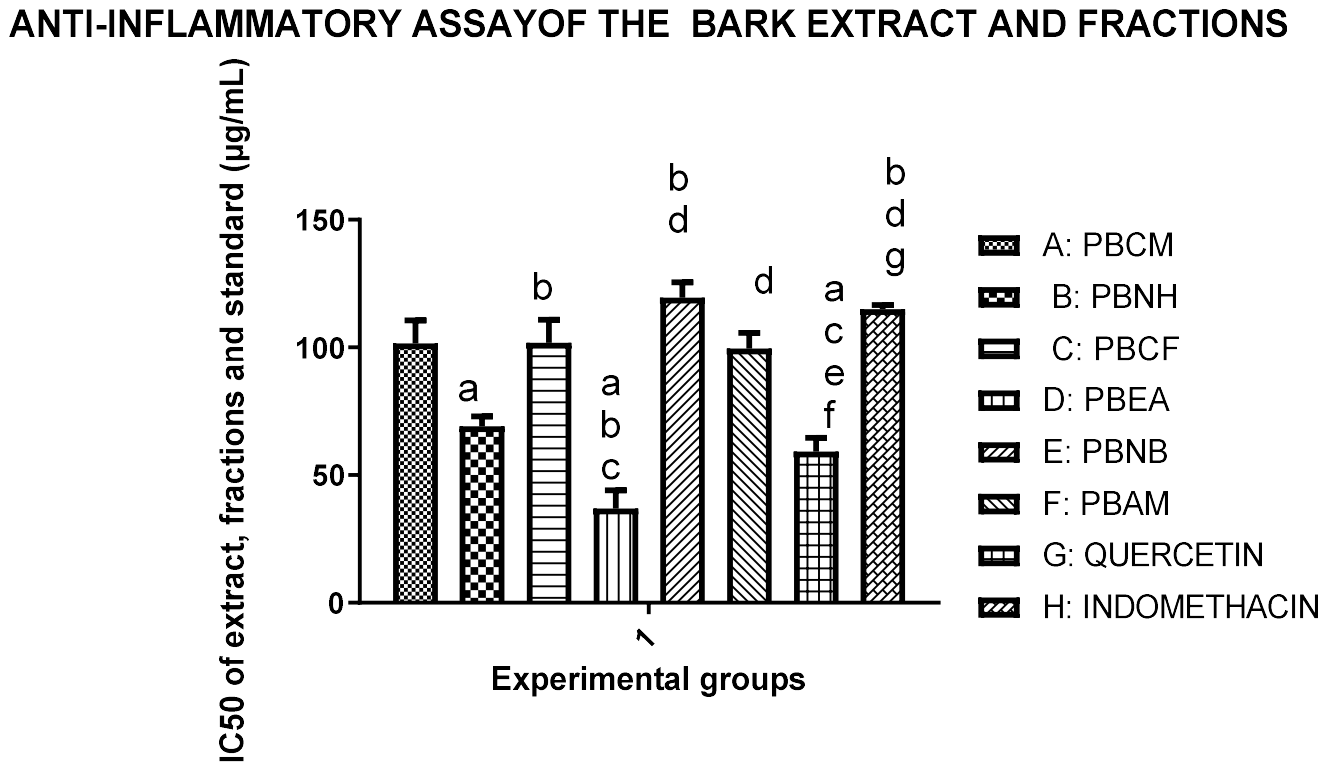


Figure 4.1.2: The IC 50 values of *P. americana* bark extract and fractions, Quercetin and Indomethacine in anti-inflammatory assay

Group A: *P. americana*bark crude methanol extract (PBCM) Group B: *P.americana* bark N-Hexane fraction (PBNH), Group C: *P.americana* bark Chloroform fraction (PBCF), Group D: *P.americana* bark Ethylacetate fraction (PBEA), Group E : *P.americana* bark N-Butanol fraction (PBNB), Group F : *P.americana* bark Aqueuos methanol fraction (PBAM), Group G : Quercetin, Group H: Indomethacin. Significance at $\alpha_{0.05.}$ Means with superscript a - h showed significance when compared with groups A - H respectively.

ANTI-HYPERTENSIVE ASSAY OF THE STEM BARK EXTRACT AND CAPTOPRIL

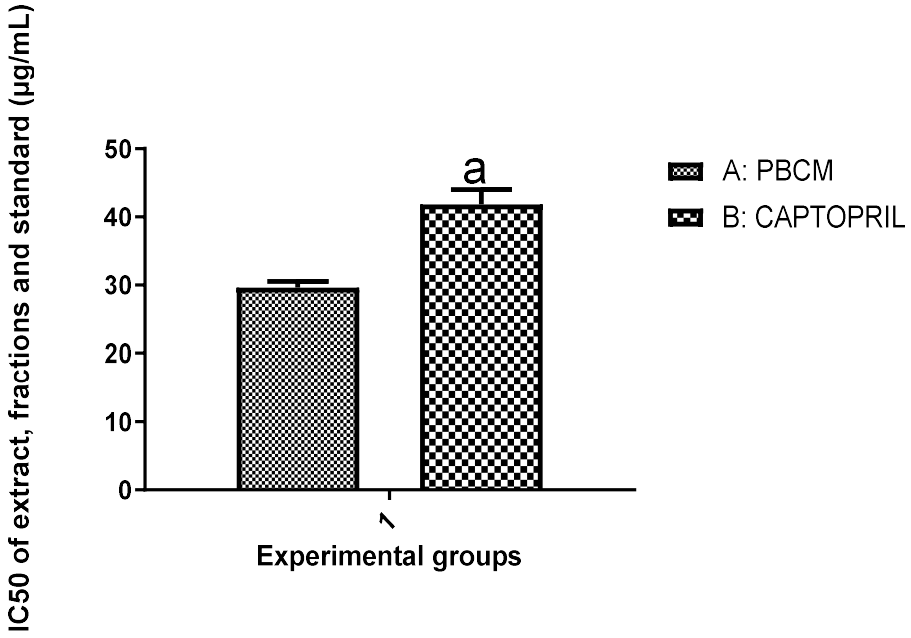


Figure 4.1.3: The IC 50 values of *P. americana* bark extract and Captopril in Anti- hypertensive assay.

Group A: *P. americana* root crude methanol extract (PRCM) Group B: Captopril.

Significance at $\alpha_{0.05.}$ Theanti-hypertensive activity of PBCM was significantly ($\alpha_{0.05}$) higher than that of the captopril.

Table 4.1.6: The IC 50 values of *P. americana* root extract and fractions and gallic acid in *invitro* biological assays

EXTRACT/ FRACTIONS	MEAN±STANDARD ERROR OF MEAN				
	ABTS ASSAY	DPPH ASSAY	HYDROXYL ASSAY	METAL CHELATING ASSAY	N. OXIDE ASSAY
A:PRCM	22.71±2.08	115.82±0.21	111.39±32.14	85.08±13.60	306.38±772.63
B:PRNH	218.72±31.24[a]	419.69±5.44[a]	25.44±4.10	16.94±2.56[a]	120.66±19.17
C:PRCF	286.67±18B.34[a]	140.46±0.35[ab]	196.42±57.80[b]	17.41±2.91[a]	182.76±36.67
D:PREA	314.47±11.31[a]	219.72±1.19[abe]	251.26±12.80[ab]	84.13±2.58[bc]	565.60±47.21
E:PRNB	22.37±2.43[bcd]	91.43±0.09[abcd]	248.90±19.01[ab]	19.91±5.63[ad]	377.44±48.97
F:PRAM	2.87±0.72[bcd]	96.62±0.13 [abcd]	149.27±20.46	80.21±30.57[bce]	110.73±13.96
G:GALLIC ACID	129.69±41.94[acdef]	113.57±6.64[bcdef]	58.71±1.94[cde]	41.92±4.39	220.29±12.38

Group A: *P. americana* root crude methanol extract (PRCM) Group B: *P.americana* root N-Hexane fraction (PRNH), Group C: *P.americana* root Chloroform fraction (PRCF), Group D: *P.americana* root Ethylacetate fraction (PREA), Group E: *P.americana* root N-Butanol fraction (PRNB), Group F: *P.americana* root Aqueous methanol fraction (PRAM), Group G : Gallic acid (GA). Significance at $\alpha_{0.05.}$ Means with superscript a - g.showed significance when compared with groups A - G. respectively.

Table 4.1.7: The OD values of *P. americana* root extract, fractions and gallic acid in Reducing power assay.

CONC.	A: PRCM	B: PRNH	C: PRCF	D: PREA	E :PRNB	F: PRAM	G: GA
15.63	0.32±0.017	0.287±0.018	0.53±0.036	0.44±0.011	0.38±0.034	0.514±0.036	0.46±0.046
31.25	0.424±0.035	0.33±0.016	0.65±0.062	0.61±0.037	0.43±0.020	0.6±0.029	0.504±0.083
62.50	0.49±0.006	0.39±0.0397	0.72±0.037	0.72±0.031	0.65±0.037	0.64±0.030	0.746±0.037
125	0.644±0.049	0.52±0.033	1.08±0.032[a]	1.119±0.16[a]	0.76±0.026	0.81±0.145	1.048±0.019[a]
250	0.739±0.11	0.75±0.312	1.124±0.06[a]	1.154±0.06[a]	0.899±0.110	1.22±0.049[a]	1.3250±0.06[a]

Group A: *P. americana* root crude methanol extract (PRCM) Group B: *P.americana* root N-Hexane fraction (PRNH), Group C: *P.americana* root Chloroform fraction (PRCF), Group D: *P.americana* root Ethylacetate fraction (PREA), Group E: *P.americana* root N-Butanol fraction (PRNB), Group F: *P.americana* root Aqueuos methanol fraction (PRAM), Group G: Gallic acid (GA). Superscripsts a - g show that the groups were significant when compared with groups A - G respectively. Significance at $\alpha_{0.05.}$

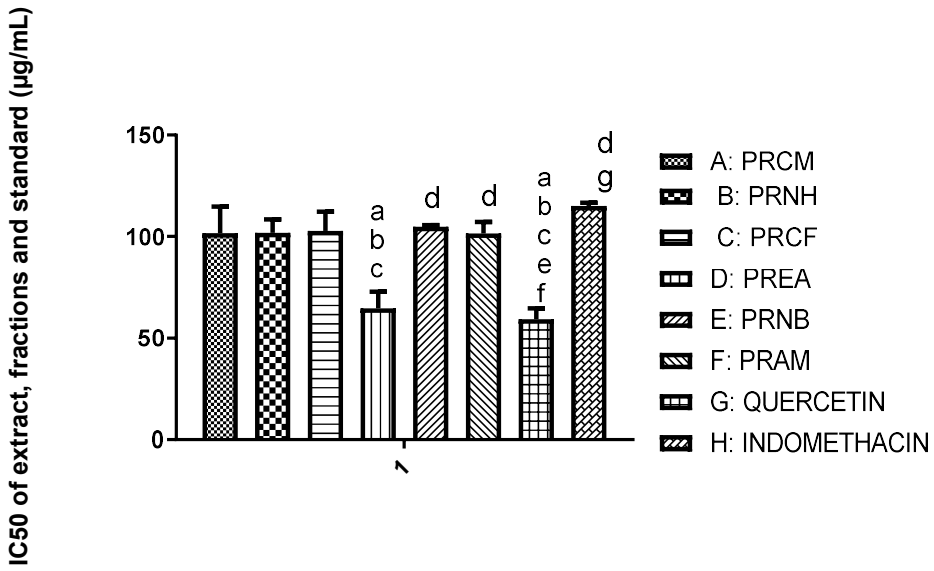


Figure 4.1.4: The IC 50 values of *P. americana* root extract, fractions, Quercetin and Indomethacine in Anti-inflammatory assay.

Group A: *P. americana* root crude methanol extract (PRCM) Group B: *P.americana* root N-Hexane fraction (PRNH), Group C: *P.americana* root Chloroform fraction (PRCF), Group D: *P.americana* root Ethylacetate fraction (PREA), Group E: *P.americana* root N-Butanol fraction (PRNB), Group F: *P.americana* root Aqueous methanol fraction (PRAM),Group G: Quercetin, Group H: Indomethacin. Superscripsts a - h show that the groups were significant when compared with groups A - H respectively. Significance at $\alpha_{0.05}$

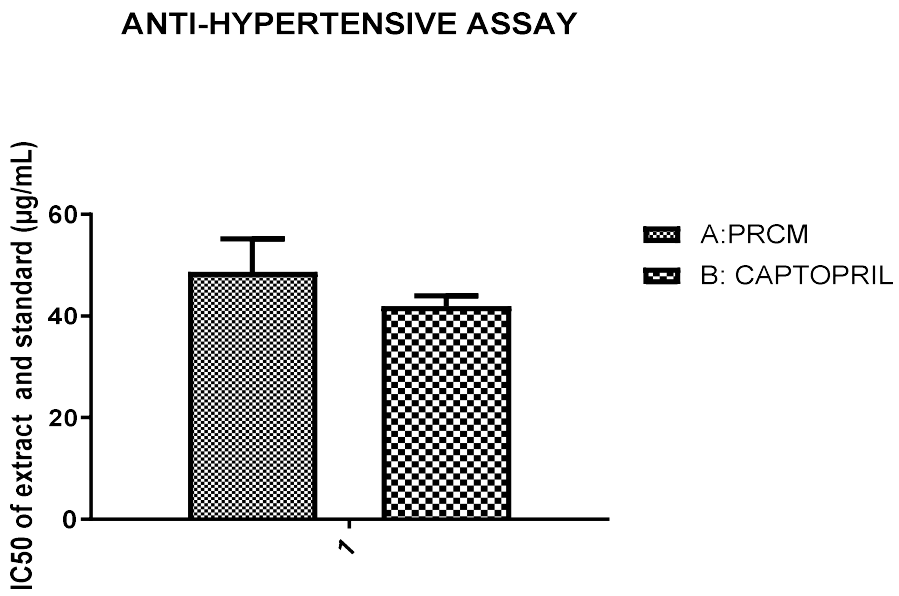


Figure 4.1.5: The IC 50 values of *P. americana* root extract and Captopril in Anti- hypertensive assay.

Group A: *P. americana* root crude methanol extract (PRCM) Group B: Captopril.

Significance at $\alpha_{0.05.}$ There was no significant difference($\alpha_{0.05}$) in the antihypertensive activities of PRCM when compared with captopril.

4.2 ACUTE TOXICITY STUDY RESULTS

4.2.0 *Persea americana* leaf extract results.

4.2.1 Median Lethal dose (LD_{50})

Signs of acute toxicity were not seen in the groups of rats given 500–2,000 mg kg^{-1} (i.e. groups B-C) However, signs of toxicity like exhaustion, incoordination, inappetence and feebleness were noticed in the group given 4,000 mg kg^{-1} but death was found in the group dosed with 5,000 mg kg^{-1}. The morbidity and mortality of 50 % seen in the group showed that the extract was toxic and lethal to rats at the dose and beyond. Therefore, the highest safe dose and lowest fatal doses for*P.americana* leaf extract were 2,000 and 5,000 mg kg^{-1}respectively'

$LD_{50} = \sqrt{2000} \text{ X } \sqrt{5000}$.

$LD_{50=} 44.721 \text{ X} 70.711$

$LD_{50 =} 3162.27$ mg kg^{-1}.

4.2.1 Blood Pressure (mmHg) of Rats in Acute Toxicity Study of leaf.

Statistically non-significant ($\alpha_{0.05}$) decreases were observed in the systolic blood pressure (SBP) across the groups with *Persea americana* leaves extract. There were dose dependent decreases in the SBP of groups B (132.67 ± 7.67), C (111.67 ± 15.76) and D (109.33 ± 4.91) when compared to the control (139.33 ± 12.91). The decreases observed in the systolic blood pressure of groups E (120.33 ± 7.31) and F (126.67 ± 9.56) however did not follow the same trend of dose dependence with those of groups A (139.33 ± 12.91), B (132.67 ± 7.67), C (111.67 ± 15.76) and D (109.33 ± 4.91) as the systolic pressure started increasing again at group E (120.33 ± 7.31) (Table 4.2.1).

There were statistically non-significant ($\alpha_{0.05}$) decreases in the diastolic blood pressure (DBP) across the treatment groups; B (111.33 ± 6.44), C (85.00 ± 14.74), D (85.33 ± 2.85), E (94.67 ± 9.40), F (103.67 ± 9.56) and G (94.33 ± 8.82) when compared to the control; A (120.00 ± 12.34), although there was no remarkable pattern in the decreases between groups (Table 4.2.1).

There were statistically non-significant decreases ($\alpha_{0.05}$) in the MAP of groups B (117.33 ± 6.89), C (93.33 ± 15.25), D (88.00 ± 7.37), E (103.67 ± 8.41), F (111.00 ± 9.29) and G (102.00 ± 9.07) compared to the control (126.33 ± 12.67). The observed decreases were dose dependent except for groups E (103.67 ± 8.41), F (111.00 ± 9.29) and G (102.00 ± 9.07) with the proceeding groups (Table 4.2.1).

Table 4.2.1Blood Pressure (mmHg) of rats in acute toxicity study of *P.americana*leaf.

	Systolic	Diastolic	Mean Arterial Pressure (MAP)
(A) Control	139.33 ± 12.91[a]	120.00 ± 12.34[a]	126.33 ± 12.67[a]
(B) PALE1000 mg kg^{-1}	132.67 ± 7.67[a]	111.33 ± 6.44[a]	117.33 ± 6.89[a]
(C) PALE 2000 mg kg^{-1}	111.67 ± 15.76[a]	85.00 ± 14.74[a]	93.33 ± 15.25[a]
(D) PALE 4000 mg kg^{-1}	109.33 ± 4.91[a]	85.33 ±2.85[a]	88.00 ± 7.37[a]
(E) PALE 5000 mg kg^{-1}	120.33 ± 7.31[a]	94.67 ± 9.40[a]	103.67 ± 8.41[a]
(F) PALE 6000 mg kg^{-1}	126.67 ± 9.56[a]	103.67 ± 9.56[a]	111.00 ± 9.29[a]
(G) PALE 7500mg kg^{-1}	119.00 ± 9.64[a]	94.33 ± 8.82[a]	102.00 ± 9.07[a]

Means with the same letter along the column are not significantly different (DMRT at $\alpha_{0.05}$)

PALE: *Persea americana* leaf extract (dosage in mg/kg)

4.2.2 The organ weights (bwt) and percentage difference in body weight of acute toxicity study of the leaf extract

The percentage (%) difference in bwt between the time of commencement and termination of the experiment were 18.3%, 18.0%, 11.7%, 15.3%, 15.2%, 9.0% and 6.3% in groups A, B, C, D, E, F and G, respectively. as shown in table 4.2.1. This shows a dose dependent progressive decrease in the percentage weight gained between the experiment period (day 0 and day 5) across the groups except in group C (Table 4.2.2).

The ratios of the weight of the heart, liver and kidneys of the rats across the groups when compared to the average body weight across the groups were normal: the organs were not hypertrophied (Table 4.2.2).

There were significant decreases ($\alpha_{0.05}$) in the weight of the left kidney of groups C (0.32 ± 0.03), D (0.34 ± 0.02), E (0.34 ± 0.003), F (0.32 ± 0.02) and G (0.32 ± 0.05) when compared to the control (0.45 ± 0.05). There was also a decrease, though not significant ($P>0.05$), in the weight of the left kidney of group B (0.44 ± 0.003) when compared to that of the control (0.45 ± 0.05) (Table 4.2.2).

There were no substantial differences ($\alpha_{0.05}$) in the weight of the right kidney across the groups.

There were substantial decreases ($\alpha_{0.05}$) in the weight of both kidneys of groups C (0.65 ± 0.04), D (0.69 ± 0.04) and G (0.56 ± 0.03) compared to the control group (0.84 ± 0.05). There was significant decrease in the weight of both kidneys of group G (0.56 ± 0.03) when compared to groups C (0.65 ± 0.04) and D (0.69 ± 0.04) (Table 4.2.2).

Table 4.2.2: Percentage change in the average weight of rats between the experimental periods.

Group	Average body weight on day 0	Average body weight on day 5	% difference in weight btw. the experimental time
A PALE (Control)	100 g	118.33 g	18.3%
B PALE (1000 mg kg^{-1})	113 g	133.33 g	18.0 %
C PALE (2000 mg kg^{-1})	103 g	115.00 g	11.7 %
D PALE (4000 mg kg^{-1})	107 g	123.33 g	15.3 %
EPALE (5000 mg kg^{-1})	110 g	126.67 g	15.2 %
F PALE (6000 mg kg^{-1})	107 g	116.67 g	9.0 %
G PALE (7500 mg kg^{-1})	95 g	101.67 g	6.3 %

PALE: *Persea americana* leaf extract (Dosage in mg/kg)

4.2.3Haematology Results of the Acute Toxicity Study of the Leaf Extract

The PCV across the groups were not significantly ($\alpha_{0.05}$) different from one another. Group B (38.67 ± 4.84) had the lowest PCV compared to the control (47.00 ± 1.73). This value was still within normal range (32-40%). There were dose reliant rise in the PCV of groups B (38.67 ± 4.84), C (41.33 ± 4.26), D (45.33 ± 2.33) and E (48.50 ± 0.50) but a reduction was observed in groups F (45.25 ± 2.10) and G (41.67 ± 5.61). Only the PCV of group E (48.50 ± 0.50) increased when compared to the control (47.00 ± 1.73). (Table 4.2.3)

There were no substantial differences ($\alpha_{0.05}$) in the haemoglobin (Hb) values in all the groups. Group B (6.13 ± 0.72) had the lowest haemoglobin (Hb) concentration compared to the control (7.27 ± 0.18). There was a dose dependent increase in the haemoglobin (Hb) concentration of groups B (6.13 ± 0.72), C (6.90 ± 0.57), D (7.37 ± 0.44) and E (7.50 ± 0.10). The haemoglobin concentration of groups F (7.10 ± 0.37) and G (6.47 ± 0.74) did not follow the trend as they had slightly lower values compared to the proceeding groups. The haemoglobin (Hb) concentration of group D (7.37 ± 0.44) and E (7.50 ± 0.10) were greater when compared with the control group (7.27 ± 0.18) (Table 4.23).

There were no substantial changes ($\alpha_{0.05}$) in the red blood cell (RBC) count across the groups. The standard physiological range for RBC count is 5.79-7.41 X 10^6 /mm3 (Spring 1998). Group B (6.13 ± 0.72) had the lower RBC count relative to the control (7.27 ± 0.18). There were dose dependent increases in the RBC counts of groups B (6.13 ± 0.72), C (6.90 ± 0.57), D (7.37 ± 0.44) to E (7.50 ± 0.10) after which there was a decrease in groups F (7.10 ± 0.37) and G (6.47 ± 0.74). The RBC count of groups D (7.37 ± 0.44) and E (7.50 ± 0.10) were higher when compared to that of the control (7.27 ± 0.18) (Table 4.23).

There were no substantial changes ($\alpha_{0.05}$) in the white blood cell (WBC) counts across all the groups. Yet, a reduction in the WBC count was observed in group B (6800.00 ± 472.58) compared to the control, group A (8133.33 ± 1017.08). However, increases were observed in groups C (8366.67 ± 726.48), D (12333.33 ± 2034.15), E (11000.00 ± 400.00), F (11350.00± 1583.51) and G (9466.67 ± 2684.73) when compared to the control group (8133.33 ± 1017.08). The pattern in the changes of the WBC counts across the groups showed a dose dependent increase from groups B (6800.00 ±

472.58), C (8366.67 ± 726.48) to group D (12333.33 ± 2034.15). Thereafter, the WBC counts decreased progressively from group E (11000.00 ± 400.00), F (11350.00± 1583.51) to G (9466.67 ± 2684.73) (Table 4.2.3).

There were no substantial changes ($\alpha_{0.05}$) in the platelet counts in all the groups.

Yet, the platelet counts of groups C (764000.00 ± 9609.02), D (804000.00 ± 107034.26), and F (717750.00 ±111019.05) were higher than that of the control while that of groups B (625000.00 ± 60893.35), E (581000.00 ± 55000.00) and G (717750.00 ±111019.05) were lower than that of the control. The observed changes were not dose reliant (Table 4.2.3).

There were no statistically substantial changes ($\alpha_{0.05}$) in the number of lymphocytes across the groups. There was no change in the lymphocyte count in group B (49.33 ± 0.33) when compared to group A. Decreases were however observed in groups C (47.83 ± 5.93) and G (44.33 ± 6.17) and increases in groups B (49.33 ± 0.33), D (49.67 ± 1.67), E (50.50 ± 1.50) and F (52.50 ± 0.87). There were dose reliant increases in groups C (47.83 ± 5.93), D (49.67 ± 1.67), E (50.50 ± 1.50) and F (52.50 ± 0.87) (Table 4.2.3).

There were no significant differences ($\alpha_{0.05}$) in the neutrophil counts across the groups. There pattern of differences however showed a slight dose dependent decrease along groups B (44.67 ± 1.86), C (43.33 ± 2.85), D (41.00 ± 1.00), E (40.50 ± 0.50) and F (40.00 ± 0.91). Neutrophil counts were higher in groups B (44.67 ± 1.86), C (43.33 ± 2.85) and G (45.67 ± 4.81) but lower in groups D (41.00 ± 1.00), E (40.50 ± 0.50) and F (40.00 ± 0.91) when compared to the control group (41.67 ± 1.67) (Table 4.2.4).

Statistically non-significant differences ($\alpha_{0.05}$) were observed in the monocyte counts across the groups. The monocytes count in groups B (5.33 ± 1.45), C (6.67 ± 2.03) and F (7.00 ± 0.71) were lower when compared to the control (8.33 ± 1.67) while those of groups D (9.33 ± 0.67) and E (9.00 ± 1.00) were higher when compared to the control group (8.33 ± 1.67). The monocyte count of group G (8.33 ± 0.88) was the same when compared to that of the control (8.33 ± 1.67). A dose dependent increase was observed from groups B (5.33 ± 1.45), C (6.67 ± 2.03) to D (9.33 ± 0.67) while a

decrease was seen in groups E (9.00 ± 1.00) when compared to F (7.00 ± 0.71) (Table 4.2.4).

There were statistically non-significant differences ($\alpha_{0.05}$) in the eosinophil counts across the groups; A (1.00 ± 1.00), B (0.67 ± 0.67), C (2.67 ± 1.45), D (0.00 ± 0.00), E (0.00 ± 0.00), F (0.50 ± 0.50) and G (1.67 ± 0.88) (Table 4.2.4).

There were a statistically non-significant difference ($\alpha_{0.05}$) in the mean corpuscular volume (MCV) across the groups. The MCV of groups B (62.67 ± 0.67), C (59.33 ± 1.67), D (61.67 ± 1.76), E (64.50 ± 0.50), F (62.75 ± 0.85) and G (64.00 ± 2.00) were slightly lower when compared to control (64.67 ± 1.86) (Table 4.2.5).

There was a statistically non-significant difference ($\alpha_{0.05}$) in the mean corpuscular haemoglobin (MCH) across the groups. There was no remarkable pattern of change in the MCH across the groups but there was an increase in the MCH of the respective group D (18.33 ± 0.33), E (19.00 ± 0.00), F (18.25 ± 0.25) and G (18.33 ± 0.88) when compared with the control group (18.00 ± 0.00). The MCH of group C (17.33 ± 0.67) was slightly lower than the control (18.00 ± 0.00) while that of group B (18.00 ± 0.00) had the same value as the control (18.00 ± 0.00) (Table 4.2.4).

There were statistically non-significant ($\alpha_{0.05}$) differences in the mean corpuscular haemoglobin concentration (MCHC) along the groups. The MCHC of groups C (29.33 ± 0.33), D (29.67 ± 0.67), E (29.00 ± 0.00), F (29.25 ± 0.75) and G (29.00 ± 0.88) was higher when compared to the control group (28.33 ± 0.88) while that of group B (28.00 ± 0.00) was slightly lower when comparedwith that of the control (28.33 ± 0.88) (Table 4.2.4).

Table 4.2.3: The haematology of Wistar rats in acute toxicity study of the leaf extract.

Group	Mean ± Standard error					
	PCV	Hb	RBC	WBC	Platelet	Lymphocyte
A)Control	47.00 ± 1.73[a]	7.27 ± 0.18[a]	7.27 ± 0.18[a]	8133.33 ± 1017.08[a]	701333.33 ± 104811.47[a]	49.00 ± 1.00[a]
B)PALE 1000 mgkg^{-1}	38.67 ± 4.84[a]	6.13 ± 0.72[a]	6.13 ± 0.72[a]	6800.00 ± 472.58[a]	625000.00 ± 60893.35[a]	49.33 ± 0.33[a]
C)PALE 2000 mgkg^{-1}	41.33 ± 4.26[a]	6.90 ± 0.57[a]	6.90 ± 0.57[a]	8366.67 ± 726.48[a]	764000.00 ± 9609.02[a]	47.83 ± 5.93[a]
D) PALE4000 mgkg^{-1}	45.33 ± 2.33[a]	7.37 ± 0.44[a]	7.37 ± 0.44[a]	12333.33 ± 2034.15[a]	804000.00 ± 107034.26[a]	49.67 ± 1.67[a]
E)PALE 5000 mgkg^{-1}	48.50 ± 0.50[a]	7.50 ± 0.10[a]	7.50 ± 0.10[a]	11000.00 ± 400.00[a]	581000.00 ± 55000.00[a]	50.50 ± 1.50[a]
F) PALE 6000 mgkg^{-1}	45.25 ± 2.10[a]	7.10 ± 0.37[a]	7.10 ± 0.37[a]	11350.00± 1583.51[a]	717750.00 ±111019.05[a]	52.50 ± 0.87[a]
G)PALE 7500 mgkg^{-1}	41.67 ± 5.61[a]	6.47 ± 0.74[a]	6.47 ± 0.74[a]	9466.67 ± 2684.73[a]	657333.33 ± 89856.06[a]	44.33 ± 6.17[a]

Means with the same superscript along the column indicates no substantial changes (DMRT at $\alpha_{0.05}$)

PALE: *Persea americana* leaf extract (Dosage: mg/kg)

Group A: Control, B: PALE 1000 Group C: PALE 2000, Group D PALE 4000, Group E: PALE 5000, Group F: PALE 6000,Group G:PALE 7500.

Table 4.2.4: The haematology of Wistar rats in acute toxicity study of the leaf extract.

	Neutrophil	Monocyte	Eosin	MCV	MCH	MCHC
A) Control	41.67 ± 1.67[a]	8.33 ± 1.67[a]	1.00 ± 1.00[a]	64.67 ± 1.86[a]	18.00 ± 0.00[a]	28.33 ± 0.88[a]
B) PALE 1000 $mgkg^{-1}$	44.67 ± 1.86[a]	5.33 ± 1.45[a]	0.67 ± 0.67[a]	62.67 ± 0.67[a]	18.00 ± 0.00[a]	28.00 ± 0.00[a]
C) PALE 2000 $mgkg^{-1}$	43.33 ± 2.85[a]	6.67 ± 2.03[a]	2.67 ± 1.45[a]	59.33 ± 1.67[a]	17.33 ± 0.67[a]	29.33 ± 0.33[a]
D) PALE 4000 $mgkg^{-1}$	41.00 ± 1.00[a]	9.33 ± 0.67[a]	0.00 ± 0.00[a]	61.67 ± 1.76[a]	18.33 ± 0.33[a]	29.67 ± 0.67[a]
E) PALE 5000 $mgkg^{-1}$	40.50 ± 0.50[a]	9.00 ± 1.00[a]	0.00 ± 0.00[a]	64.50 ± 0.50[a]	19.00 ± 0.00[a]	29.00 ± 0.00[a]
F) PALE 6000 $mgkg^{-1}$	40.00 ± 0.91[a]	7.00 ± 0.71[a]	0.50 ± 0.50[a]	62.75 ± 0.85[a]	18.25 ± 0.25[a]	29.25 ± 0.75[a]
G) PALE 7500 $mgkg^{-1}$	45.67 ± 4.81[a]	8.33 ± 0.88[a]	1.67 ± 0.88[a]	64.00 ± 2.00[a]	18.33 ± 0.88[a]	29.00 ± 0.88[a]

Means with the same superscript along the column indicates no substantial changes (DMRT at $\alpha_{0.05}$)

PALE: *Persea americana* leaf extract (Dosage: $mgkg^{-1}$)

4.2.4 Serum Chemistry Results of the Acute Toxicity Study of the Leaf Extract

Table 4.2.5: The biochemical parameters of the serum of Wistar rats in the acute toxicity study of methanol extract of *P. americana*leaf.

GROUP	ALP	ALT	AST	BUN	CREAT	HDL
A) Control	112.00±9.34	30.00±0.82	39.50±3.12	16.60±0.89	0.68±0.05	19.93±1.18
B) 1000 $mgkg^{-1}$	110.50±5.43	34.33±0.52	47.83±2.64[a]	16.90±1.08	0.80±0.21[a]	25.6±0.84[a]
C) 2000 $mgkg^{-1}$	104.00±9.06	30.50±1.00	41.75±1.26	16.40±0.71[a]	0.83±0.12[a]	23.02±2.27[a]
D) 4000 $mgkg^{-1}$	107.67±13.05	30.00±1.73	41.00±1.73[b]	17.53±0.91	0.87±0.12[a]	23.17±1.63[a]
E) 5000 $mgkg^{-1}$	100.00±17.69	35.00±1.00	50.33±2.31[a]	17.93±0.31[a]	1.00±0.20[a]	24.77±0.72[a]
F) 6000 $mgkg^{-1}$	112.50±8.19	34.00±1.41[a]	48.50±4.65[a]	16.50±1.49	0.85±0.10[a]	22.85±1.72[a]
G) 7500 $mgkg^{-1}$	112.00±12.00	28.00±2.00	39.33±2.08[a]	17.33±0.96	0.77±0.05[a]	20.00±2.07[a]

Superscript (a) indicates a significant increase compared with control at $\alpha_{0.05}$.

PALE: *Persea americana* leaf extract (Dosage: $mgkg^{-1}$)

Group A: Control, B: PALE 1000 mg kg^{-1}, Group C: PALE 2000 mg kg^{-1},Group D: PALE 4000 mg kg^{-1},Group E: PALE 5000 mg kg^{-1}, Group F: PALE 6000mg kg^{-1}Group G:PALE 7500 mg kg^{-1}.

4.2.5 Histology Slides for Acute Toxicity Study of the leaf Extract.

4.2.5.1 Cardiac Tissues

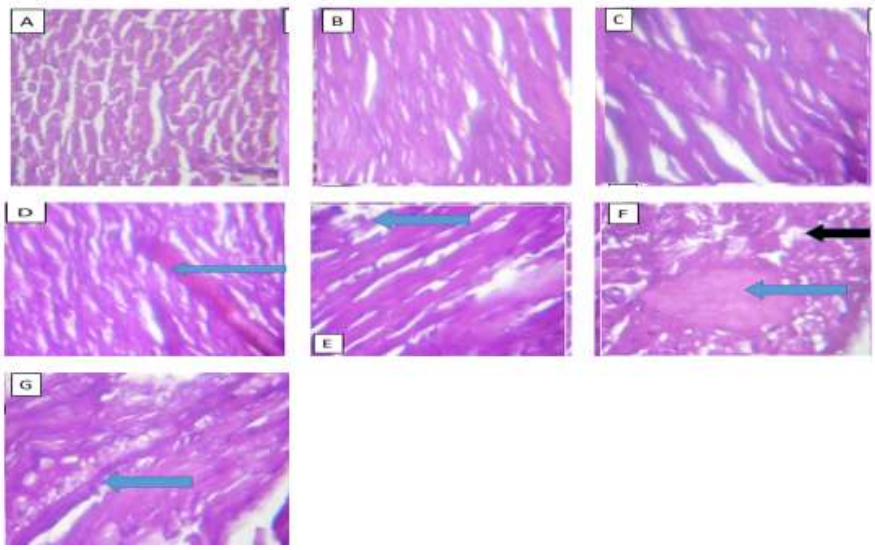


Figure 4.2.1: Histology slides of heart of rats (Groups A-G) in acute toxicological studies on methanolic *P. americana* leaf extract.

There was non-significant lesion in groups A, B and C. Group D: Heart section shows congestion of blood vessels (Blue arrow). Group E: Slide shows mild necrotic areas (Blue arrow). Group F: Slide shows focal areas of thrombosis occluding blood vessels (Blue arrow) and necrotic areas (Black arrow). Group G: There was hypercellularity of the mesangial cells of the glomeruli as well as merging of the glomeruli to the Bowman's capsule (blue arrow).

4.2.5.2 Renal Tissues

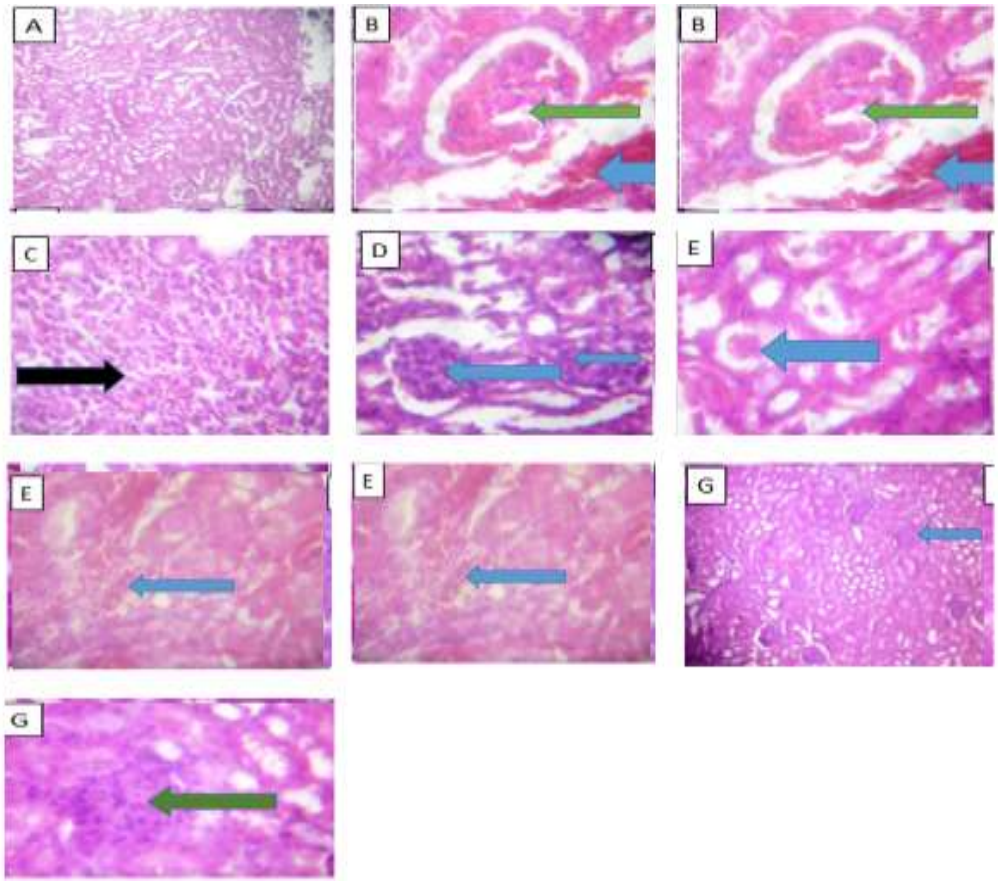
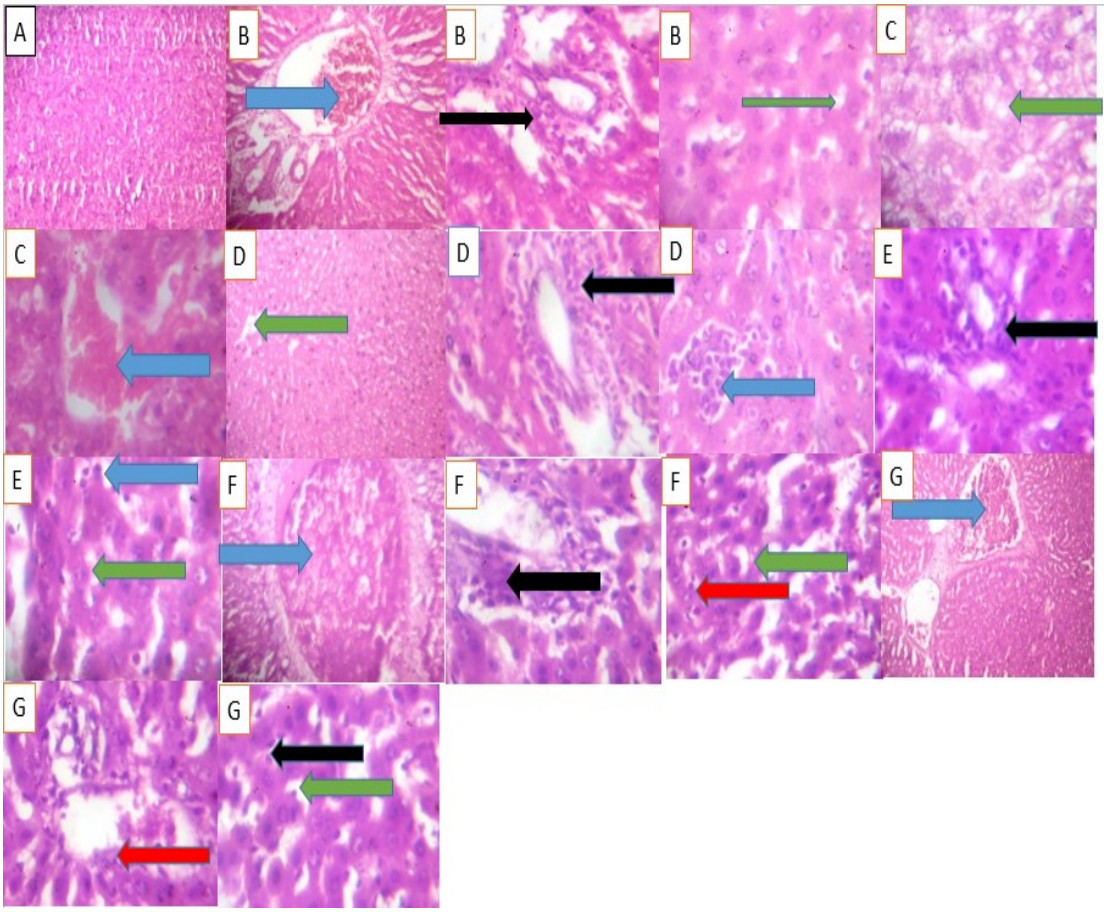


Figure 4.2.2: Histology slides of kidney of rats (Groups A-G) in acute toxicological studies on methanolic *P. americana*leaf extract.

Group A: Slide shows no significant lesions. Group B: Plate shows mild disseminated congestion of blood vessels (Blue arrow). The glomerulus also appears congested (Green arrow). Group C: Plates show focal areas of renal casts (green arrows) and focal areas of marked interstitial infiltration by inflammatory cells (blue arrow). Group D: Plate shows hypercellularity of the mesangial cells of the glomeruli (blue arrows). Group E: Plates show focal area of inflammation (bolder blue arrow), and red cell cast (blue arrow). Group F: Plate shows hypercellularity of mesangial cells of the glomeruli as well as merging of the glomeruli and the Bowman's capsule (blue arrow). Group G: there was hypercellularity of the mesangial cells of the glomeruli (blue arrow) and merging of the glomeruli to the Bowman's capsule (green arrow).



4.2.5.3: Liver Tissues

Figure 4.2.3: Histology slides of liver of rats (Groups A-G) in acute toxicological studies on methanolic *P. americana* leaves extract.

Group A: There was no remarkable lesion. Group B: Plates show congestion of vessels (blue arrow), mild inflammation of the periportal area (black arrow) and mild disseminated macro and micro vesicular steatosis (green arrows). Group C: There was intravascular congestion (blue arrow), and moderate disseminated macro and micro vesicular steatosis (green arrows). Group D: Plates show mild periportal inflammation (black arrow), focal area of lymphoid aggregate as well as zone 2 mild inflammatory cells infiltration. (blue arrow) and mild disseminated macro and micro vesicular steatosis (green arrow).Group E: Plates show congestion of blood vessels, disseminated steatosis (green arrow), mild infiltration of zone 2 (slender arrow) and inflammation of the periportal (black arrow). Group F: Plates show congestion of vessels (blue arrow), very mild inflammation of the periportal area (black arrow), mild infiltration of zone 2 by inflammatory cells (red arrow) and mild disseminated macro vesicular steatosis (green arrow). Group G: Plates show intravascular congestion (blue arrows), very mild periportal inflammation (red arrow), mild infiltration of zone 2 by inflammatory cells (black arrow) and mild disseminated micro vesicular steatosis (green arrows).

4.2.6 Results of the Acute Toxicity study of *P.americana* bark.

4.2.6.0 Acute Toxicity study and Median lethal dose calculation.

Signs of acute toxicity were not seen in the groups of rats administered 1000–2,000 mg kg^{-1} (i.e. A and B) However, symptoms of acute toxicity like fatigue, exhaustion, lack of coordination, anorexia and feableness were evident in those given 4,000 mg kg^{-1}. I found dead rats in the groups administered 6,000 mg kg^{-1}. The mortality seen in the group showed that the extract was toxic and lethal to rats at the dose of 5,000 mg kg^{-1} and beyond. Therefore, the highest safe dose and lowest fatal dose for*P.americana*bark extract were 2,000 and 6,000 mg kg^{-1}, respectively

Median lethal dose (LD_{50}) of the PA bark was as below:

$LD_{50} = \sqrt{2,000} \text{ X } \sqrt{6,000}$.

$LD_{50} = 44.721 \text{ X} 77.46$

$LD_{50} = 3463.91 \text{mg kg}^{-1}$

4.2.7 The Organ weights and Percentage changes in the weight of rats.

The percentage (%) changes in the average body weight between the period of experiment goes thus; -26.1%, -11.3%, 2.2%, 3.8%, 1.8%, 7.8%, 10.5% in groups A, B, C,D ,E, F, and G respectively as shown in (Table 4.3.5). This shows an initial percentage weight loss in Group A and B followed by percentages of weight increase in group C, D, E, F, and G.

The ratios of the weight of the heart, liver and kidneys across the groups compared to the average weight of the rats across the groups were normal. The organs were not hypertrophied as illustrated in (Table 4.2.6).

There was a statistically remarkable decrease ($\alpha_{0.05}$) in the weight of the heart of group F (0.40 ± 0.00^{c}) when compared to the control (0.48 ± 0.05^{a}).

The weights of the kidneysignificantly increased ($\alpha_{0.05}$) in groups B (0.95 ± 0.03^{a}), C (1.03 ± 0.08^{a}), D (0.98 ± 0.09^{ab}) when compared to the control (0.75 ± 0.03^{c}).

There were no significant differences ($\alpha_{0.05}$) in the weights of the kidney across the groups.

The weights of the Liver of the groups C (6.38 ± 0.35^{a}) and D (5.73 ± 0.45^{ab}) increased significantly when compared with the control (4.63 ± 0.12^{c}).

The weights of the kidney of the groups E (0.90 ± 0.10^{bc}) and F (0.80 ± 0.00^{bc}) increased significantly when compared with the control group(0.75 ± 0.03^{c})

Table 4.2.6: Percentage difference in the average body weights from the onset to the end of experiment periods in the acute toxicity of *P.americana bark*.

Group	Average body weight on day 0	Average body weight on day 5	% difference in weight between the time of experiment
A (Control)	186.63	138.00	-26.1%
B (PA 1000 mg kg^{-1})	183.30	162.50	-11.3%
C (PA 2000 mg kg^{-1})	177.33	181.25	2.2%
D (PA 4000 mg kg^{-1})	173.38	180.00	3.8%
E (PA 5000 mg kg^{-1})	162.13	165.00	1.8%
F(PA 6000 mg kg^{-1})	143.85	155.00	7.8%
G (PA 7500 mg kg^{-1})	137.20	151.67	10.5%

PABE: *Persea americana* bark extract (Dosage in mg/kg)

Group A: Control, B: PABE 1000 mg kg^{-1}, Group C: PABE 2000 mg kg^{-1},Group D: PABE 4000 mg kg^{-1},Group E: PABE 5000 mg kg^{-1}, Group F: PABE 6000mg kg^{-1}Group G:PABE 7500 mg kg^{-1}.Significance was measured at $\alpha_{0.05}$, Means with superscript a, b, c, d, e, f, g showed significance when compared with groups A, B, C, D, E, F, G, respectively.

Table 4.2.7: Body and organ weights of wistar rats in Acute Toxicity Test of *P. americana*root

Group	Mean ± Standard error			
	Body weight	**Heart**	**Kidney**	**Liver**
A (Control)	138.00 ± 4.91^{c}	0.48 ± 0.05^{a}	0.75 ± 0.03^{c}	4.63 ± 0.12^{c}
B (PA 1000 mg kg^{-1})	162.50 ± 3.23^{abc}	0.65 ± 0.03^{ab}	0.95 ± 0.03^{a}	4.98 ± 0.11^{bc}
C (PA 2000mg kg^{-1})	181.25 ± 11.61^{a}	0.65 ± 0.10^{ab}	1.03 ± 0.08^{a}	6.38 ± 0.35^{a}
D (PA 4000 mg kg^{-1})	180.00 ± 7.36^{ab}	0.60 ± 0.04^{abc}	0.98 ± 0.09^{ab}	5.73 ± 0.45^{ab}
E (PA 5000 mg kg^{-1})	165.00 ± 10.00^{abc}	0.70 ± 0.01^{a}	0.90 ± 0.10^{bc}	5.15 ± 0.45^{bc}
F(PA 6000 mg kg^{-1})	155.00 ± 7.64^{abc}	0.40 ± 0.01^{c}	0.80 ± 0.01^{bc}	4.63 ± 0.23^{c}
G (PA 7500 mg kg^{-1})	151.67 ± 3.02^{bc}	0.53 ± 0.07^{abc}	0.93 ± 0.03^{abc}	5.00 ± 0.32^{bc}

Means with the same letter along the column are not significantly different (DMRT at $p<.05$)

PABE: *Persea americana* bark extract (Dosage in mg/kg)

Group A: Control, B: PABE 1000 mg kg^{-1}, Group C: PABE 2000 mg kg^{-1},Group D: PABE 4000 mg kg^{-1},Group E: PABE 5000 mg kg^{-1}, Group F: PABE 6000mg kg^{-1}Group G:PABE 7500 mg kg^{-1}.

4.2.8 Blood Pressure (mmHg) of Acute Toxicity Study of the Bark Extract.

Statistically significant decreases ($\alpha_{0.05}$) were observed in the systolic blood pressure measurements of group B (155.00 ± 4.16^{b}), E (148.00 ± 7.51^{b}) and F (158.00 ± 15.59^{b}), when compared to the control (160.00 ± 4.36^{a}). However, a statistically significant ($\alpha_{0.05}$) increase in the systolic pressure of group C (188.67 ± 26.69^{ab}), D (206.00 ± 2.52^{a}), G (163.67 ± 4.63^{b}) when compared to the control (160.00 ± 4.36^{a}) when compared respectively (Table 4.2.8).

There was a statistically non-significant reduction ($\alpha_{0.05}$) in the diastolic blood pressure in group B (108.33 ± 2.40^{b}), when compared to the control group (109.00 ± 13.05^{b}). There was a statistically remarkable increase ($\alpha_{0.05}$) in the diastolic blood pressure in group D (165.67 ± 5.61^{a}) and a statistically non-significant increases ($\alpha_{0.05}$) in the diastolic blood pressure in groups C (133.67 ± 4.33^{ab}), E (117.00 ± 10.69^{b}), F (117.00 ± 21.73^{b}), G (137.67 ± 5.46^{ab}), when compared to the control group (109.00 ± 13.05^{b}) (Table 4.2.8).

Statistically non-significant decreases ($\alpha_{0.05}$) was observed in the mean arterial pressure in groups B (123.67 ± 0.88^{a}), C (151.33 ± 9.39^{a}), D (179.00 ± 3.36^{a}), E (127.00 ± 9.61^{a}), F (132.00 ± 17.52^{a}), G (145.33 ± 2.96^{a}) (Table 4.2.8).

Table 4.2.8. Blood Pressure (mmHg) of wistar rats in acute toxicity study to *P. americana*bark.

	Mean ± Standard error		
Group	**Systolic**	**Diastolic**	**Mean Arterial Pressure (MAP)**
(A) Control	160.00 ± 4.36[a]	109.00 ± 13.05[b]	190.33 ± 60.34[a]
(B) PA500 mg/kg	155.00 ± 4.16[b]	108.33 ± 2.40[b]	123.67 ± 0.88[a]
(C) PA1000 mg/kg	188.67 ±26.69[ab]	133.67 ± 4.33[ab]	151.33 ± 9.39[a]
(D) PA 2000 mg/kg	206.00 ± 2.52[a]	165.67 ± 5.61[a]	179.00 ± 3.36[a]
(E) PA 4000 mg/kg	148.00 ± 7.51[b]	117.00 ± 10.69[b]	127.00 ± 9.61[a]
(F) PA 6000 mg/kg	158.00 ± 15.59[b]	117.00 ± 21.73[b]	132.00 ± 17.52[a]
(G) PA 7500 mg/kg	163.67 ± 4.63[b]	137.67 ± 5.46[ab]	145.33 ± 2.96[a]

Means with the same letter along the column are not significantly different (DMRT at $p<.05$)

PABE: *Persea americana* bark extract (Dosage in mg/kg)

Group A: Control, B: PABE 1000 mg kg^{-1}, Group C: PABE 2000 mg kg^{-1},Group D: PABE 4000 mg kg^{-1},Group E: PABE 5000 mg kg^{-1}, Group F: PABE 6000mg kg^{-1}Group G:PABE 7500 mg kg^{-1}.

4.2.9 Haematology result of acute toxicity study of *P. americana* bark

There were statistically significant differences ($\alpha_{0.05}$) in the PCV across the groups. Besides, there were increases across the groups B (45.00 ± 2.00^{a}), C (41.00 ± 1.23^{a}), D (43.75 ± 2.96^{a}) E (47.50 ± 0.50^{a}), F (43.33 ± 1.67^{a}) and G (41.33 ± 3.84^{a}), when compared with the control (40.00 ± 1.16^{a}) (Table 4.2.9).

There were no statistically significant differences ($\alpha_{0.05}$) in the Hb across the groups. However, there were increases in groups B (14.86 ± 0.69^{a}), C (13.48 ± 0.38^{a}), D(14.58 ± 0.99^{a}) E (15.55 ± 0.05^{a}), F (14.13 ± 0.42^{a}), G (14.50 ± 1.90^{a}), when compared with the control group (13.27 ± 0.39^{a} (Table 4.2.9).

There were no statisticallysignificant differences ($\alpha_{0.05}$) in the RBC across the groups. Besides, across the group there were an increase from group B (7.45 ± 0.39^{a}), C (6.79 ± 0.22^{a}), D (7.18 ± 0.59^{a}) E (7.96 ± 0.24^{a}), F (7.23 ± 0.23^{a}), G (7.01 ± 0.60^{a}), when compared with the control group (6.71 ± 0.24^{a}) (Table 4.2.9).

There were no statisticallysignificant differences ($\alpha_{0.05}$) in the WBC across the groups. However there was a decrease in group B (2810.00 ± 444.52^{a}) followed by increases in group C (4062.50 ± 154.62^{a}), D (5737.50 ± 1404.22^{a}) E (6800.00 ± 300.00^{a}), F (9476.67 ± 916.90^{a}), G (5283.33 ± 308.67^{a}), when compared with the control group (4033.33 ± 88.19^{a}) (Table 4.2.9).

There were no statistically significant differences ($\alpha_{0.05}$) in the platelet across the groups. However, there were decreases in group B ($251200.00 \pm 18254.86^{a}$) C ($260000.00 \pm 26012.82^{a}$), D ($291250.00 \pm 50572.35^{a}$) and G ($296333.33 \pm 27870.73^{a}$) while there was an increase in E ($338000.00 \pm 42000.00^{a}$), and F ($327000.00 \pm 154993.94^{a}$), when compared with the control ($302,000.00 \pm 12489.99^{a}$) (Table 4.2.9).

There were no statistically significant differences ($\alpha_{0.05}$) in the lymphocyte across the groups. Besides, across the group there were decreases in groups C (66.75 ± 1.65^{a}) F (67.33 ± 4.84^{a}), G (67.33 ± 5.90^{a}) while there were increases in B (73.40 ± 2.73^{a}), D (71.00 ± 1.58^{a}) and E (69.00 ± 1.00^{a}), when compared with the control group (68.33 ± 3.76^{a}) (Table 4.2.10).

There were no statistically significant differences ($\alpha_{0.05}$) in the Neutrophil across the groups. Besides, there were decreases in groups B (23.40 ± 2.54^{a}), D (25.75 ± 1.83^{a}), F (27.00 ± 4.51^{a}) while there were increases in C (28.50 ± 1.66^{a}), E (27.50 ± 0.50^{a} and G (28.67 ± 6.01^{a}), when compared with the control group (27.00 ± 5.20^{a}) (Table 4.2.10).

There were no statistically significant difference ($P > 0.05$) in the Monocyte across the groups. Besides, there were decreases in groups B (1.80 ± 1.45^{a}), C (1.50 ± 2.29^{a}), D (1.50 ± 0.29^{a}) E (0.00 ± 0.00^{a}), F (2.33 ± 0.33^{a}), G (1.67 ± 0.33^{a}), when compared with the control (2.67 ± 1.67^{a}) (Table 4.2.10).

There were no statistically significant differences ($\alpha_{0.05}$) in the eosinophil cross the groups. However, there were decreases in group B (0.67 ± 0.67^{a}), D (0.01 ± 0.00^{a}), E (0.01 ± 0.00^{a}) and F (0.50 ± 0.50^{a}) while there were increases in C (2.67 ± 1.45^{a}), and G (1.67 ± 0.88^{a}), when compared with the control group (1.00 ± 1.00^{a}) (Table 4.2.10).

Table 4.2.9: Haematology result showing the erythrocyte parameters of albino rats in acute toxicity study of the bark extract.

Groups	Mean ± Standard error			
	PCV	**Hb**	**RBC**	**Platelet**
(A) Control	40.00 ± 1.16[a]	13.27 ± 0.39[a]	6.71 ± 0.24[a]	302000.00±12489.99[a]
(B) PABE 1000 mg kg^{-1}	45.00 ± 2.00[a]	14.86 ± 0.69[a]	7.45 ± 0.39[a]	251200.00 ± 8254.86[a]
(C) PABE 2000 mg kg^{-1}	41.00 ± 1.23[a]	13.48 ± 0.38[a]	6.79 ± 0.22[a]	260000.00 ±26012.82[a]
(D) PABE 4000 mg kg^{-1}	43.75 ± 2.96[a]	14.58 ± 0.99[a]	7.18 ± 0.59[a]	291250.00 ±50572.35[a]
(E) PABE 5000 mg kg^{-1}	47.50 ± 0.50[a]	15.55 ± 0.05[a]	7.96 ± 0.24[a]	338000.00 ± 42000.00 [a]
(F) PABE 6000 mg kg^{-1}	43.33 ± 1.67[a]	14.13 ± 0.42[a]	7.23 ± 0.23[a]	327000.0 ±154993.94[a]
(G) PABE 7500 mg kg^{-1}	41.33 ± 3.84[a]	14.50 ± 1.90[a]	7.01 ± 0.60[a]	296333.33 ± 27870.73 [a]

PABE: *Persea americana* bark extract (Dosage is in mg/kg)

Table 4.2.10: Haematology result showing the leucocyte parameters of Albino rats in acute toxicity study of methanol extract of *Persea americana* bark.

Groups	**Mean ± Standard error**			
	WBC	**Neutrophil**	**Monocyte**	**Eosinophil**
(A) Control	4033.33 ± 88.19[a]	27.00 ± 5.20[a]	2.67 ± 1.67[a]	1.00 ± 1.00[a]
(B) PABE 1000 mg kg^{-1}	2810.00 ± 44.52[a]	23.40 ± 2.54[a]	1.80 ± 1.45[a]	0.67 ± 0.67[a]
(C) PABE 2000 mg kg^{-1}	4062.50±154.62[a]	28.50 ± 1.66[a]	1.50 ± 2.29[a]	2.67 ± 1.45[a]
D) PABE 4000 mg kg^{-1}	5737.50 ±1404.22[a]	25.75 ± 1.83[a]	1.50 ± 0.29[a]	0.01 ± 0.00[a]
E) PABE 5000 mg kg^{-1}	6800.00 ± 300.00[a]	27.50 ± 0.50[a]	1.50 ± 0.50[a]	0.01 ± 0.00[a]
F) PABE 6000 mg kg^{-1}	9476.67± 916.90[a]	27.00 ± 4.51[a]	2.33 ± 0.33[a]	0.50 ± 0.50[a]
G) PABE 7500 mg kg^{-1}	5283.33 ± 308.67[a]	28.67 ± 6.01[a]	1.67 ± 0.33[a]	1.67 ± 0.88[a]

Means with the same letter along the column are not significantly different (DMRT at $\alpha_{0.05}$)

PABE: *Persea americana* bark extract

4.2.10 Haematology result of acute toxicity study of *P. americana* bark

There was statistically significant difference ($P < 0.05$) in the ALT values in group G (34.5±0.71) when compared with group A (31.50±0.71). The differences in groups B (32.50±0.71), C (34.30±1.55), D (31.50±0.71), E (32.61±2.31) and F (34±1.41) were not significant ($P > 0.05$) when compared with the control group (31.50±0.707).

There was statistically significant difference ($P < 0.05$) in the AST values in groups B (47.5±0.71), D (42.5±0.71), E (47±1.00), F (46.5±0.70), and G (48.5±2.12) when compared to the control group (40.5±0.71), Group C (46±2.82) however was not statistically significant ($P > 0.05$) when compared with the control.

There was statistically significant difference ($P < 0.05$) in the BUN values in groups C (17.20±1.39), D (14.90±0.42), when compared to the control A (14.20±0.71). Groups B (15.82±0.78), E (16.60±0.53), F (46.5±0.71), G (15.35±1.20) were however not significant ($P > 0.05$) when compared with the control group.

There was statistically significant difference ($P < 0.05$) in the Creatinine values in groups C (0.9±0.1732), F (0.81±0.07), G (0.78±0.001) when compared with group A (0.55±0.07). Groups B (0.8±.17), D (0.67±0.06), E (0.75±0.072), were, however, not significant ($P > 0.05$) when compared with group A (0.55±0.07)

Table 4.2.11: Serum Biochemistry result of Albino rats in acute toxicity study of methanol extract of *Persea americana* bark.

Groups	**Mean± Standard deviation**			
	ALT	**AST**	**BUN**	**Creatinine**
(A) Control	31.50±0.707	40.5±0.707	14.20±0.71	0.55±0.0707
(B) PA 200 mg/kg	32.50±0.707	47.5±0.707^{a**}	15.82±0.78	0.8±.1732
(C) PA 500 mg/kg	34.30±1.55	46±2.828	17.20±1.386^{a*}	0.9±0.1732^{a*}
(D) PA 100 mg/kg	31.50±0.71	42.5±0.707^{b**}	14.90±0.424^{a*}	0.67±0.058
(E) PA 4000 mg/kg	32.61±2.31	47±1.0^{a**}	16.60±0.53	0.75±0.072
(F) PA 5000 mg/kg	34±1.41	46.5±0.707^{a**}	16.35±1.0607	0.81±0.072^{a*}
(G) PA 7500 mg/kg	34.5±0.71^{a*}	48.5±2.121^{a*}	15.35±1.202	0.78±0.001^{a*}

PABE: *Persea americana* bark extract (mg kg^{-1})

Group A: Control, B: PABE 1000, Group C: PABE 2000,Group D: PABE 4000, Group E: PABE 5000, Group F: PABE 6000,Group G: PABE 7500.Significance was measured at $\alpha_{0.05}$, Means with superscript a-g showed significance when compared with groups A- G, respectively.

4.2.10 Results of the Acute Toxicity Study of the *P. americana* Root extract.

4.2.10.0 Acute Toxicity Studies and Median lethal dose of the *P. americana* root extract

4.2.10.1 Calculation of Median Lethal dose (LD_{50})

The groups of rats dosed 1000–4,000 mg kg−1 did not manifest signs of acute toxicity, whereas signs of acute toxicity such as abnormal gait, loss of coordination, anorexia, and feebleness were observed in the rats dosed 5,000 mg kg−1 but death was recorded in rats administered 7,500 mg kg−1 when compared to the control group. Therefore, the maximum safe dose and the minimum lethal dose of *P.americana*bark extract were 4,000 and 7,500 mg kg^{-1}, respectively

Median lethal dose for the root extract was:

$LD_{50} = \sqrt{4,000} \text{ X } \sqrt{7,500}.$

$LD_{50} = 63.245 \text{ X} 86.605$

$LD_{50} = 5477.23 \text{mg kg}-1$

4.2.11 Haematological parameters.

Table 4.2.12 Haematology parameters of rats in acute toxicity study of *P. americana* root extract

Group	Mean ± Std. deviation					
	PCV%	Hb(g/dl)	WBC(10^9/L)	Neutrophil (10^9/L)	Lymphocyte(10^9/L)	Monocyte (10^9/L)
A	51.33 ± 4.73	16.93 ± 1.61	12700.00 ± 7036.33	20.33 ± 4.16	69.67 ± 9.29	8.00 ± 1.73
B	50.00 ± 2.58	16.53 ± 0.95	11075.00 ± 861.68	19.50 ± 1.73	71.25 ± 3.69	8.50 ± 1.00
C	49.00 ± 1.83	16.18 ± 0.78	13200.00 ± 2011.63	21.00 ± 4.55	69.75 ± 9.22	7.00 ± 2.45
D	48.00 ± 3.56	15.93 ± 1.23	12000.00 ± 1651.26	17.50 ± 3.79	73.75 ± 7.89	7.50 ± 1.92
E	52.67 ± 4.93	17.47 ± 1.62	13833.33 ± 2967.04	19.67 ± 4.62	70.67 ± 9.24	8.00 ± 1.73
F	51.33 ± 3.06	17.13 ± 0.96	14200.00 ± 2920.62	17.33 ± 2.52	75.67 ± 3.06	7.00 ± 1.00
G	52.33 ± 4.73[b]	17.67 ± 1.21	15066.67 ± 5052.06	16.67 ± 0.58	76.00 ± 1.73	7.33 ± 1.16

PARE: *Persea americana* root extract

Group A: Control, B: PARE 1000 mg kg^{-1}, Group C: PARE 2000 mg kg^{-1},Group D: PARE 4000 mg kg^{-1},Group E: PARE 5000 mg kg^{-1}, Group F: PARE 6000mg kg^{-1}Group G:PARE 7500 mg kg^{-1}.

Table 4.2.13: Other haematology parameters of rats in acute toxicity study of *P. americana* root extract.

Group	Mean ± Std. deviation		
	Eosin(10^9/L)	**Basophil(10^9/L)**	**Platelets(10^9/L)**
A	1.00 ± 1.73	1.00 ± 1.73	918000.00 ± 480076.04
B	0.75 ± 1.50	0.00 ± 0.00	879500.00 ± 94747.03
C	1.75 ± 2.06	0.50 ± 0.50	1045750 ± 345719.90
D	1.25 ± 2.50	0.00 ± 0.00	1313000. ± 429202.36
E	1.33 ± 2.31	0.33 ± 0.58	981666.67 ± 196291.45
F	0.00 ± 0.00	0.00 ± 0.00	1066666.7 ± 345328.58
G	0.00 ± 0.00	0.00 ± 0.00	1200666.7 ± 308216.37

Values are expressed as mean±standard variation. Significance is at ($\alpha_{0.05}$)

PARE: *Persea americana* root extract (mg/kg)

Group A: Control, B: PARE 1000, Group C: PARE 2000,Group D: PARE 4000,Group E: PARE 5000, Group F: PARE 6000, Group G:PARE 7500 mg kg^{-1}.

Table 4.2.14: Serum chemistry analytes of rats in acute toxicity study of the *P. americana* root extract

Group	Mean ± Std. deviation						
	AST(units/L)	ALT (units/L)	ALP (units/L)	UREA(mmol/L)	Creatinine (mg/dL)	CHOL	HDL
A	39.00 ± 2.00[a]	29.00 ± 2.16[a]	122.25 ± 5.73[a]	16.50 ± 1.16[a]	0.63 ± 0.10[a]	76.33 ± 2.31[a]	27.00 ± 5.48[a]
B	43.00 ± 3.08[a]	32.20 ± 2.17[a]	121.00 ± 6.67[a]	14.98 ± 4.85[a]	0.74 ± 0.05[a]	74.00 ± 0.00[a]	28.20 ± 1.64[a]
C	38.50 ± 3.11[a]	29.50 ± 2.65[a]	117.50 ± 4.80[a]	16.60 ± 1.28[a]	0.58 ± 0.10[a]	74.00 ± 0.00[a]	25.00 ± 3.56[a]
D	39.50 ± 3.54[a]	29.50 ± 2.12[a]	114.00 ± 4.24[a]	17.10 ± 0.71[a]	0.60 ± 0.14[a]	67.50 ± 2.50[a]	29.00 ± 5.66[a]
E	38.00 ± 2.83[a]	26.00 ± 1.41[a]	116.50 ± 4.95[b]	16.50 ± 0.71[b]	0.55 ± 0.07[a]	67.50 ± 2.50[a]	30.00 ± 0.00[a]
F	42.00 ± 2.94[a]	30.50 ± 2.65[a]	125.25 ± 2.63[a]	16.93 ± 0.97[a]	0.73 ± 0.10[a]	71.00 ± 0.00	24.00 ± 3.65[a]
G	40.25 ± 2.21[a]	28.25 ± 3.59[a]	119.00 ± 5.77[a]	16.25 ± 0.64[a]	0.63 ± 0.10[a]	71.00 ± 0.00	25.50 ± 5.44[a]

Means with the same letter along the column are not significantly different (DMRT at $\alpha_{0.05}$)

PARE: *Persea americana* root extract (mg/kg)

Group A: Control, B: PARE 1000, Group C: PARE 2000, Group D: PARE 4000, Group E: PARE 5000, Group F: PARE6000, Group G:PARE 7500 mg kg^{-1}.

There were non-significant ($\alpha_{0.05}$) dose dependent decreases in PCV of groups B, (50.00 ± 2.58), C (49.00 ± 1.83), D (48.00 ± 3.56), compared to A (51.33 ± 4.73). There were non-significant increases in PCV in groups E, (52.67 ± 4.93), F (51.33 ± 3.06), G (52.33 ± 4.73) compared to A (51.33 ± 4.73).There was a non-significant ($\alpha_{0.05}$) increase in group G (52.33 ± 4.73) compared to group B (50.00 ± 2.58) (Table 4.2.11).

There were non-significant ($\alpha_{0.05}$) dose dependent decreases in heamoglobin of groups B, (16.53 ± 0.95), C, (16.18 ± 0.78), D (15.93 ± 1.23) compared to group A (16.93 ± 1.61). There were non-significant ($\alpha_{0.05}$) increases in groups E, (17.47 ± 1.62), F (17.13 ± 0.96), G (17.67 ± 1.21) (Table 4.2.11).

There were non-significant ($\alpha_{0.05}$) increases in white blood cells counts of groups C (13200.00 ± 2011.63), D (12000.00 ± 1651.26), E (13833.33 ± 2967.04), F (14200.00 ± 2920.62), G (15066.67 ± 5052.06) compared to group B (11075.00 ± 861.68). There was also a decrease in group B (11075.00 ± 861.68) compared to the control group A (12700.00 ± 7036.33) (Table 4.2.11).

There were non-significant ($\alpha_{0.05}$) decreases in neutrophils counts of groups B, (19.50 ± 1.73), D (17.50 ± 3.79), E (19.67 ± 4.62), F (17.33 ± 2.52), G (16.67 ± 0.58) compared to group A (20.33 ± 4.16). But there was non-significant ($\alpha_{0.05}$) increase in group C compared to other groups (Table 4.2.11).

There were non-significant ($\alpha_{0.05}$) increases in lymphocytes counts in groups B (71.25 ± 3.69), C (69.75 ± 9.22), D (73.75 ± 7.89), E (70.67 ± 9.24), F (75.67 ± 3.06), G (76.00 ± 1.73) compared to group A (69.67 ± 9.29) (Table 4.2.11).

There were non-significant ($\alpha_{0.05}$) decreases in monocytes counts of group A (8.00 ± 1.73) compared to group B (8.50 ± 1.00). There were non-significant ($\alpha_{0.05}$) decreases of monocytes in group C (7.00 ± 2.45), D (7.50 ± 1.92), E (8.00 ± 1.73), F (7.00 ± 1.00), G (7.33 ± 1.16) compared to group B (8.50 ± 1.00) (Table 4.2.11).

There was a non-significant increase ($\alpha_{0.05}$) in eosinophils count in groups A (1.00 ± 1.73) compared to group B (0.75 ± 1.50). There were non-significant ($\alpha_{0.05}$) increases in eosinophil counts of groups C (1.75 ± 2.06), D (1.25 ± 2.50), E (1.33 ± 2.31) compared to group B (0.75 ± 1.50) (Table 4.2.12).

There was a non-significant ($\alpha_{0.05}$) increase in platelets counts in group A (918000.00 ± 480076.04) compared to group B (879500.00 ± 94747.03). There were non-significant ($\alpha_{0.05}$) increases in platelets countsof groups C (1045750.00 ± 345719.90), D (1313000.00 ± 429202.36), E (981666.67 ± 196291.45), F (1066666.67 ± 345328.58), G (1200666.67 ± 308216.37) compared to group B (879500.00 ± 94747.03) (Table 4.2.12).

There was also non-significant ($\alpha_{0.05}$) decreases in AST enzyme of group A (39.00 ± 2.00) compared to group B (43.00 ± 3.08). There were also non-significant ($\alpha_{0.05}$) decreases in AST enzyme of groups C (38.50 ± 3.11), D (38.00 ± 2.83), E (42.00 ± 2.94), F (40.25 ± 2.21) compared to group B (43.00 ± 3.08) (Table 4.2.13).

There was non-significant ($\alpha_{0.05}$) increases in ALT enzymelevel in group B (32.20 ± 2.17) compared to group A (29.00 ± 2.16). There were non-significant ($\alpha_{0.05}$) decreases of ALT enzyme level of groups C (29.50 ± 2.65), D (29.50 ± 2.12), E (26.00 ± 1.41), F (30.50 ± 2.65), G (28.25 ± 3.59) compared to group B (32.20 ± 2.17) (Table 4.2.13).

There was a non-significant ($\alpha_{0.05}$) decrease in ALP enzyme level of group B (121.00 ± 6.67) compared to group A (122.25 ± 5.73). There were non-significant decreases ($\alpha_{0.05}$) of ALP enzyme of groups C (117.50 ± 4.80), D (114.00 ± 4.24), E (116.50 ± 4.95), F (125.25 ± 2.63), G (119.00 ± 5.77) compared to group A (122.25 ± 5.73). There was non-significant ($\alpha_{0.05}$) increase of ALP in group F (125.25 ± 2.63) compared to B (121.00 ± 6.67) (Table 4.2.13).

There was a non-significant ($\alpha_{0.05}$) decreases in urea of group B (14.98 ± 4.85) compared to group A (16.50 ± 1.16). There were also a non-significant ($\alpha_{0.05}$) increases in group C (16.60 ± 1.28), D (17.10 ± 0.71), E (16.50 ± 0.71), F (16.93 ± 0.97), G (16.25 ± 0.64) compared to in group B (14.98 ± 4.85) (Table 4.2.13).

There were anon-significant ($\alpha_{0.05}$) increase in creatinine of groups B (0.74 ± 0.05) and F (0.73 ± 0.10) when compared withgroup A (0.63 ± 0.10). There were also non-significant ($\alpha_{0.05}$) decreases in creatinine of group C (0.58 ± 0.10), D (0.60 ± 0.14), E (0.55 ± 0.07), G (0.63 ± 0.10) when compared withgroup A (0.63 ± 0.10) (Table 4.2.13).

4.2.11.1 Blood Pressure Measurements

Table 4.2.15: Blood pressure of rats in acute toxicity Study of *P.americana* root extracts

	Mean ± Std. deviation		
Group	**Systole**	**Diastole**	**MAP**
A	123.33 ± 19.04	99.00 ± 36.29	107.33 ± 29.26^{d}
B	133.00 ± 7.94	88.67 ± 23.44^{d}	103.00 ± 16.46^{d}
C	145.33 ± 18.45	106.67 ± 11.59	119.33 ± 11.59
D	140.33 ± 23.35	105.67 ± 23.07	116.67 ± 22.37
E	198.25 ± 17.41^{ab}	153.00 ± 14.02^{ab}	174.50 ± 26.39^{ab}
F	223.00 ± 22.65^{a}	180.67 ± 12.50^{a}	194.00 ± 12.77^{a}
G	187.00 ± 11.27^{b}	133.67 ± 32.35^{b}	154.00 ± 23.64^{bc}

PARE: *Persea americana* bark extract. Blood pressure is measured in mmHg

Group A: Control, B: PARE 1000 mgkg^{-1}, Group C: PARE 2000 mgkg^{-1}, Group D PARE 4000 mgkg^{-1}, Group E: PARE 5000 mgkg^{-1}, Group F: PARE 6,000 mgkg^{-1},Group G: PARE 7500 mgkg^{-1}.Significance was measured at $\alpha_{0.05}$, Means with superscript a - g showed significance when compared with groups A - G, respectively.

There was a non-significant ($\alpha_{0.05}$) increase in systolic blood pressure in group B (133.00 ± 7.94) compared to group A (123.33 ± 19.04). There was a significant increase ($\alpha_{0.05}$) in systolic blood pressure in group E (198.25 ± 17.41) compared to group A (123.33 ± 19.04) and B (133.00 ± 7.94). There was a significant ($\alpha_{0.05}$) increase in systolic blood pressure in group F (223.00 ± 22.65) compared to group A (123.33 ± 19.04). There was a significant ($\alpha_{0.05}$) increase in systolic blood pressure in group G (187.00 ± 11.27) compared to group B (133.00 ± 7.94). There were statistically non-significant ($\alpha_{0.05}$) differences in the systolic blood pressure measurements of groups A (123.33 ±19.04), B (133.00 ± 7.94), C (145.33 ± 18.45), D (140.33 ± 23.35) (Table 4.2.14).

There was a significant ($\alpha_{0.05}$) decrease in diastolic blood pressure between group B (88.67 ± 23.44) compared to group D (105.67 ± 23.07). There was also a significant ($\alpha_{0.05}$) increase in diastolic blood pressure of group E (153.00 ± 14.02) compared to group A (99.00 ± 36.29) and B (88.67 ± 23.44). There was a significant ($\alpha_{0.05}$) increase in diastolic blood pressure of group F (180.67 ± 12.50) compared to group A (99.00 ± 36.29). There is a significant ($\alpha_{0.05}$) increase in diastolic blood pressure of group G (133.67 ± 32.35) compared to group A (99.00 ± 36.29). Groups A (99.00 ± 36.29), C (106.67 ± 11.59), D (105.67 ± 23.07) are not significantly different (Table 4.2.14).

There was a significant ($\alpha_{0.05}$) decrease in mean arterial pressure of groups A (107.33 ± 29.26), B (103.00 ± 16.46) compared to group E (174.50 ± 26.39). There were significant ($\alpha_{0.05}$) decreases in mean arterial pressure of groups B (103.00 ± 16.46), C (119.33 ± 11.59) compared to group G (154.00 ± 23.64). There was a significant ($\alpha_{0.05}$) decrease in mean arterial pressure of group A (107.33 ± 29.26) when compared to group F (194.00 ± 12.77). Groups C (119.33 ± 11.59), D (116.67 ± 22.37) were not significantly ($\alpha_{0.05}$) different. Groups B (103.00 ± 16.46), A (107.33 ± 29.26) were not also significantly ($\alpha_{0.05}$) different (Table 4.2.14).

4.2.12 TOTAL PHENOLIC CONTENT ASSAY OF *Persea americana*EXTRACTS AND FRACTIONS

4.2.12.1 Total Phenolic Content Assay of the *P. americana* Leaf

Table 4.2.16; Total phenolic content assay of Persea Americana leaf Extract and Fractions.

EXTRACT/FRACTIONS	TOTAL PHENOLIC COMPOUND (MEAN ± SEM)
A: PLCM	62.68±2.82
B: PLNH	31.36 ±2.95[a]
C: PLCF	41.05 ±4.97[a]
D: PLEA	58.86 ±3.79[bc]
E: PLNB	104.39 ±2.08[acd]
F: PLAM	114.12 ±1.38[abcd]

Group A: *P. americana* leaf crude methanol extract (PLCM) Group B: *P.americana* leaf N-Hexane fraction (PLNH) , Group C : *P.americana* leaf Chloroform fraction (PLCF), Group D : *P.americana* leaf Ethylacetate fraction (PLEA), Group E : *P.americana* leaf N-Butanol fraction (PLNB), Group F : *P.americana* leaf Aqueuos methanol fraction (PLAM), Group G : Significance at $\alpha_{0.05.}$ Means with superscript a - f showed significance when compared with groups A- F, respectively. The unit for TPC is mg GAE/g

4.2.12.2 Total Phenolic Content Assay of the *P. americana* Bark

Table 4.2.17: Total phenolic content assay of *Persea americana* bark Extract and Fractions

EXTRACT/FRACTIONS	TOTAL PHENOLIC COMPOUND (MEAN ± SEM)
A: PBCM	62.46 ± 1.44
B: PBNH	79.26 ±5.31
C: PBCF	115.56 ± 1.80^{ab}
D: PBEA	115.45 ± 1.79^{ab}
E: PBNB	62.13 ± 6.80^{cd}
F: PBAM	28.22 ± 2.88^{abcde}

Total phenolic content (mg GAE/g) of *P. americana* bark extract and fractions.

Group A: *P. americana*bark crude methanol extract (PBCM) Group B: *P.americana* bark N-Hexane fraction (PBNH), Group C: *P.americana* bark Chloroform fraction (PBCF), Group D: *P.americana* bark Ethylacetate fraction (PBEA), Group E :*P.americana* bark N-Butanol fraction (PBNB), Group F : *P.americana* bark Aqueuos methanol fraction (PBAM), Group G : Gallic acid (GA). Significance at $\alpha_{0.05.}$ Means with superscript a, b, c, d, e and f showed significance when compared with groups A-F, respectively.

4.2.12.3 Total Phenolic Content Assay of the *P. americana* Root.

Table 4.2.18: Total phenolic content of *P. americana* root extract and fractions

EXTRACT/FRACTIONS	TOTAL PHENOLIC COMPOUND (MEAN ± SEM)
PRCM	113.64 ±6.18
PRNH	36.85 ±14.25[a]
PRCF	78.67 ±10.97
PREA	72.13 ±3.29
PRNB	64.53 ±18.77
PRAM	119.99 ±3.02[be]

Total phenolic content (mg GAE/g) of *P. americana* root extract and fractions.

Group A: *P. americana* root crude methanol extract (PRCM) Group B: *P.americana* root N-Hexane fraction (PRNH), Group C: *P.americana* root Chloroform fraction (PRCF), Group D: *P.americana* root Ethylacetate fraction (PREA), Group E: *P.americana* root N-Butanol fraction (PRNB), Group F: *P.americana* root Aqueuos methanol fraction (PRAM), Superscripts a,b e, show that the groups were significant when compared with groups A, B and E respectively. Significance at $\alpha_{0.05.}$

4.2.13Phytochemical analysis of the P.americana root

Table 4.2.19: The Qualitative Phytochemical constituents of *P. americana root extracts.*

Serial Number	Phytochemicals	Present (P) /Absent (A)
1	Alkaloids	P
2	Phytosterols	P
3	Glycosides	P
4	Flavonoids	P
5	Phenols	P
6	Saponins	P
7	Triterpenes	P
8	Anthraquinones	A
9	Tanins	A

4.3.0 RESULTS OF THE *IN VIVO* ANTIHYPERTENSIVE ASSAY

Table 4.3.1: The Percentage body weight differences between onset and termination of dosing in the experimentally induced hypertensive rats treated with *P. americana*leaf, bark and root extracts and lisinopril.

GROUP	Average weight before experiment (g)	Average weight after experiment (g)	% Gain/ loss during experiment. (g)
AControl	110..35	126.50	14.6%
B (L-NAME)	130.62	145.00	11.01%
C(L-NAME+ PALE 1)	146.20	148.00	1.92%
D (L-NAME+ PALE 2)	171.46	173.13	0.97%
E(L-NAME+ PALE 3)	143.88	156.88	9.04%
F (L-NAME + PABE 1)	169.75	170.71	0.57%
G (L-NAME + PABE 2)	167.17 ± 3.10^{e}	202.78	21.3%
H (L. Name + PABE 3)	179.54 ± 2.36^{cd}	157.78	-12.1%
I(L-NAME+ PARE 1)	138.80	145.30	4.68 %
J (L-NAME+ PARE 2)	125. 45	130.00	3.63 %
K(L-NAME+ PARE 3)	114.36	123.15	7.69 %
L(L-NAME+ LISINOPRIL	212.43	188.89	-11.1%

L-NAME was given orally at 40mg/kg. Lisinopril was given at 0.28mg/kg and Extracts measured in mg kg^{-1}at the dosage of 100 (1), 200 (2) and 400 (3) respectively, for the*Persea americana* leaf (PALE), bark (PABE) and root (PARE) extracts as given to groups B-K with L-NAME while A received distilled water and Lreceived L. NAME + Lisinopril.

Significance was measured at values presented as mean ± SEM.Significance was measured at $\alpha_{0.05}$, Means with superscript a - l showed significance when compared with their respective groups A-L.

Table 4.3.2: Effects of *P. Americana* leaf, bark and rootextractsand lisinopril on blood pressure measurement of hypertensive rats

GROUPS	Mean ± Standard error		
	Systolic Blood Pressure (mmHg)	**Diastolic Blood Pressure (mmHg)**	**Mean Arterial pressure (mmHg)**
AControl	130.33 ± 7.23	79.67 ± 9.33	122.00 ± 11.63
B (L-NAME)	224.33 ± 11.89^{a}	170.67 ± 21.99^{a}	188.67 ± 18.62^{a}
C(L-NAME+ PALE 1)	134.50 ± 4.50^{ab}	93.00 ± 9.00^{ab}	127.50 ± 16.38^{b}
D (L-NAME+ PALE 2)	110.50 ± 31.87^{b}	100.50 ± 10.59^{ab}	123.00 ± 9.41^{b}
E(L-NAME+ PALE 3)	147.75 ± 7.791^{bcd}	104.75 ± 11.16^{ab}	110.50 ± 10.69^{be}
F (L-NAME + PABE1)	160.33 ± 17.95^{abe}	131.33 ± 12.25^{abcde}	140.67 ± 15.25^{bd}
G (L-NAME + PABE 2)	113.67 ± 9.49^{bcdf}	91.33 ± 17.84^{bf}	104.67 ± 15.17^{bf}
H (L. Name + PABE 3)	$130.33 \pm 8.9O^{bcf}$	113.67 ± 9.49^{abga}	125.67 ± 9.62^{b}
I(L-NAME+ PARE 1)	131.33 ± 14.25^{bcf}	$120.67 \pm 15.25^{abcdegh}$	134.50 ± 4.50^{bdg}
J (L-NAME+ PARE 2)	$111.33 \pm 17.84^{abcdfghi}$	104.67 ± 15.17^{abfhj}	$158.00 \pm 37.00^{abcdegh}$
K(L-NAME+ PARE 3)	113.67 ± 9.49^{bckdfj}	105.67 ± 9.62^{abegij}	127.33 ± 3.22^{bj}
L(L-NAME+ LISINOPRIL	126.33 ± 9.82^{bcfj}	$82.33 \pm 6.64^{bcdfjgihjk}$	$96.67 \pm 7.79^{abcfhijk}$

L-NAME was given orally at 40mgkg^{-1}. Lisinopril was given at 0.28mgkg^{-1} and Extracts measured in mgkg^{-1}at the dosage of 100 (1), 200 (2) and 400 (3), respectively for the*Persea americana* leaf (PALE), bark (PABE) and root (PARE) extracts as given to groups B-K with L-NAME while A received distilled water and Lreceived L. NAME + Lisinopril.

Significance was measured at values presented as mean ± SEM. Groups. Significance was measured at $\alpha_{0.05}$, Means with superscript a - l showed significance when compared withwith their respective groups A-L.

Table 4.3.3: Electrocardiogram of hypertensive rats treated with *Persea americana* leaf, bark and root extracts and Lisinopril.

	Mean ± Standard Error of Mean						
	Heart rate (bpm)	**P(ms)**	**PR (ms)**	**QRS (ms)**	**QT (ms)**	**QTc (ms)**	**Ra (mV)**
A	218.33±6.43	21.33±3.06	45±2.00	14.67±3.51	45.33±26.08	73.33±35.57	0.44±0.17
B	220.33±21.13	29.33±5.13	51.00±1.00	17.25±3.78	106.00 ± 1.00^{a}	217.00 ± 1.85^{ab}	0.56±0.07
C	208.33±33.56	18.67 ± 5.51^{b}	45±5.57	14.67±1.53	62 ± 11.53^{ab}	115.33 ± 25.79^{ab}	0.26 ± 0.03^{ab}
D	196.67±18.50	27.67 ± 1.53^{a}	47.67±3.06	14.33±1.15	70.33 ± 3.22^{ab}	131 ± 1.00^{ab}	0.58 ± 0.13^{c}
E	249.75 ± 26.04^{acd}	22.75±3.20	43.5±9.26	16±3.46	86.25 ± 1.5^{abcd}	164 ± 8.49^{abcd}	0.39 ± 0.13^{bc}
F	247.67 ± 2.08^{cd}	19.00 ± 1.00^{bd}	27.33 ± 1.53^{abcd}	5.67±4.16	76.33 ± 1.53^{abe}	138.00 ± 2.65^{abe}	0.33 ± 0.09^{bd}
G	197.00 ± 1.00^{ef}	31.00 ± 2.65^{cef}	48.00 ± 1.00^{ef}	13.00 ± 1.00^{b}	67.67 ± 11.06^{abe}	115.33 ± 24.17^{abe}	0.27 ± 0.04^{abd}
H	177.33 ± 40.28^{abcef}	23.00 ± 9.85^{g}	43.33 ± 1.53^{f}	13.33 ± 1.52^{b}	47.67 ± 2.52^{bdefg}	89.33 ± 2.08^{bcdefg}	0.26 ± 0.06^{abde}
I	176.33 ± 1.53^{abcef}	17.33 ± 1.53^{bdg}	41.67 ± 1.53^{f}	18.00 ± 1.00^{gh}	62.67 ± 1.53^{abeh}	141.3 ± 210.78^{abcgh}	0.24 ± 0.03^{abde}
J	253.33 ± 4.62^{abcdghi}	24.00±1.00	33.67 ± 17.04^{abcdg}	11.00 ± 1.00^{befi}	77.00 ± 1.00^{abchi}	135.67 ± 5.03^{abeh}	0.24 ± 0.01^{abde}
K	250.67 ± 23.97^{acdghi}	18.33 ± 0.58^{bdg}	43.00 ± 4.36^{f}	12.33 ± 2.08^{bi}	87.00 ± 2.00^{abcdghi}	167.33 ± 5.51^{abcdfghj}	0.25 ± 0.01^{abde}
L	234.67 ± 10.12^{dghi}	20.67 ± 9.50^{bg}	37.67 ± 12.42^{b}	$23.67\pm2.86^{abcdefghijk}$	86.0 ± 10.44^{abcdghi}	$169.7\pm18.82^{abcdfghij}$	0.33 ± 0.06^{bd}

L-NAME was given orally at 40 mg kg^{-1}. Lisinopril was given at 0.28mg kg^{-1}.

PALE, PABE&PARE – *Persea americana*leaf, bark and root extracts, respectively. Extracts were measured in mg kg^{-1}

Significance was measured at values presented as mean ± SEM. Group A (Control), Group B (L. NAME), Group C (L. NAME + PALE 100), Group D (L. NAME + PALE 200), Group E (L. NAME + PALE 400). F (L. NAME + PABE 100), Group G (L. NAME + PABE 200), Group H (L. NAME + PALE 400), I (L. NAME + PARE 100), Group G (L. NAME + PARE 200), Group H (L. NAME + PARE 400), Group L (L. NAME + Lisinopril). Significance was measured at $\alpha_{0.05}$, Means with superscript a- l showed significance when compared with with their respective groups A-L. HR (bpm), P, QT amd QTc segment and QRS (ms)

Table 4.3.4: Markers of oxidative stress in hypertension and the modulatory effect of *Persea americana*leaf, bark and root extracts and Lisinopril on the heart tissues of rats

GROUPS	MDA	H2O2	PC
A	0.57±0.22	40.28±4.42	1068.55±564.30
B	0.87±0.25[a]	110.1±13.9	1371.58±323.26
C	0.54±0.12[cb]	38.45±3.78	965.35±235.40
D	0.44±0.08[b]	37.51±2.86	897.65±302.21
E	0.59±0.11[b]	38.43±4.79	794.21±185.30[b]
F	0.54±0.27[b]	100.5.6±10. `91[abcdef]	940.1±185.6
G	0.53±0.14[b]	97.30±15.22[abcde]	901.50±356.5
H	0.6±0.11	99.08±10.99[abcde]	1029±122.00
I	0.42±0.24[b]	42.49±5.88[fh]	929.35±552.10
J	0.33±0.22[b]	43.58±6.32[fh]	883.87±407.41
K	0.63±0.20[j]	41.81±4.96[fh]	438.58±202.16[abch]
L	0.48±0.10[b]	40.68±3.86[fh]	1134.95±283.36

L-NAME was given orally at 40mgkg^{-1}. Lisinopril was given at 0.28mgkg^{-1} and Extracts measured in mgkg^{-1}at the dosage of 100 (1), 200 (2) and 400 (3), respectively for the*Persea americana* leaf (PALE), bark (PABE) and root (PARE) extracts as given to groups B-K with L-NAME while A received distilled water and Lreceived L. NAME + Lisinopril.

Significance was measured at values presented as mean ± SEM. Groups. Significance was measured at$\alpha_{0.05}$, Means with superscript a - l showed significance when compared with with their respective groups A-L. MDA (µl/mg protein), H2O2 (µmol/mg protein) and PC (µmol/mg protein)

Table 4.3.5: Markers of oxidative stress in hypertensiverats and the modulatory effect of *Persea americana* leaf, bark and root extracts and lisinopril on the renal tissues

GROUPS	MDA	H2O2	PC
AControl	0.60±0.11	45.84±7.61	918.6±478.7
B (L-NAME)	1.11±0.04$^{\mathbf{a}}$	51.38±12.05^{ab}	1709.0± 155.93^{a}
C(L-NAME + PALE 1)	0.51±0.09$^{\mathbf{b}}$	35.93±2.74$^{\mathbf{ab}}$	1027±418.1^{b}
D (L-NAME + PALE 2)	0.41±0.14$^{\mathbf{b}}$	32.75±1.86^{ab}	960±187.50^{b}
E(L-NAME + PALE 3)	0.50±0.28$^{\mathbf{b}}$	34.63±2.31$^{\mathbf{ab}}$	821.9±308.9^{b}
F (L-NAME + PABE1)	0.47±0.19^{b}	31.98±2.19^{ab}	1363±356.7^{ae}
G (L-NAME + PABE 2)	0.60±0.19^{b}	39.42±1.39$^{\mathbf{b}}$	832.8±137.70^{bf}
H (L. Name + PABE 3)	0.34±0.12^{ab}	35.98±2.19^{ab}	1043±381.40^{b}
I(L-NAME+ PARE 1)	0.71±0.08^{bdh}	45.51±6.71^{bcdefh}	901.08±191.98^{bf}
J (L-NAME+ PARE 2)	0.68±0.27^{bdh}	45.71±12.23^{bcdefh}	994.20±303.72^{b}
K(L-NAME+ PARE 3)	0.74±0.18^{bdfh}	47.98±6.54^{bcdefh}	643.59±152.3^{bf}
L(L-NAME+ LISINOPRIL	0.56±0.14$^{\mathbf{b}}$	34.20±2.70$^{\mathbf{abijk}}$	962.75±107.83^{b}

L-NAME was given orally at 40mgkg^{-1}. Lisinopril was given at 0.28mgkg^{-1} and Extracts measured in mgkg^{-1}at the dosage of 100 (1), 200 (2) and 400 (3), respectively, for the*Persea americana* leaf (PALE), bark (PABE) and root (PARE) extracts as given to groups B-K with L-NAME while A received distilled water and Lreceived L. NAME + Lisinopril.MDA (µl/mg protein), H2O2 (µmol/mg protein) and PC (µmol/mg protein)

Significance was measured at values presented as mean ± SEM. Groups. Significance was measured at $\alpha_{0.05}$, Means with superscript a - l showed significance when compared with with their respective groups A-L.

Table 4.3.6: Antioxidant and modulatory effects of *Persea americana*leaf, bark and root extracts and lisinopril on the heart tissue of hypertensive rats.

GROUPS	REDUCED GLUTATHIONE	NON-PROTEIN THIOL	PROTEIN THIOL
A	64.139±24.66	54.72±8.31	40.61±6.48
B	51.82±13.2^{a}	38.26±5.65^{b}	31.72±7.70
C	89.74±1.69$^{\mathbf{ab}}$	64.83±4.43^{abc}	38.78±8.08
D	88.37±1.55$^{\mathbf{abc}}$	81.57±15.38^{bd}	38.51±10.10
E	88.77±1.55^{bc}	56.96±10.72^{bd}	87.83±12.89$^{\mathbf{abcd}}$
F	54.21±13.2^{cde}	47.17±13.44^{cd}	44.25±6.84^{e}
G	73.72±11.53^{cde}	55.45±4.80^{bd}	44.16±8.44^{e}
H	57.88±13.35^{cde}	56.65±3.74^{bd}	44.41±7.17^{be}
I	65.79±8.61^{acde}	51.16±13.13^{d}	44.25±6.84^{e}
J	67.80±7.99^{cde}	55.97±10.94^{bd}	44.16±8.44^{e}
K	64.07±8.27^{bcdefgi}	54.72±8.73^{bd}	41.05±10.42^{e}
L	68.74±3.68^{bcde}	57.17±13.44^{bd}	44.44±5.20^{be}

GSH (µmol/g tissue) NPT (µmol/mg protein) PT (µmol/mg protein)

L-NAME was given orally at 40mgkg^{-1}. Lisinopril was given at 0.28mgkg^{-1} and Extracts measured in mgkg^{-1}at the dosage of 100 (1), 200 (2) and 400 (3), respectively for the*Persea americana* leaf (PALE), bark (PABE) and root (PARE) extracts as given to groups B-K with L-NAME while A received distilled water and Lreceived L. NAME + Lisinopril.

Significance was measured at values presented as mean ± SEM. Groups. Significance was measured at $\alpha_{0.05}$, Means with superscript a - l showed significance when compared with their respective groups A-L.

Table 4.3.7:Antioxidant and modulatory effects of *Persea americana*leaf, bark and root extracts and lisinopril on the renal tissue of hypertensive rats.

	REDUCED GLUTATHIONE	NON-PROTEIN THIOL	PROTEIN THIOL
A	85.36±7.71	39.99±2.89	41.05±10.42
B	54.58±7.60	13.32±5.89^{a}	30.11±9.73
C	87.02±9.71^{ab}	76.77±8.05$^{\mathbf{b}}$	40.10±14.13
D	82.62±4.53^{b}	82.80±25.98$^{\mathbf{abc}}$	41.21±9.29^{b}
E	84.15±5.75^{b}	81.19±22.22$^{\mathbf{bd}}$	37.99±9.32^{abcd}
F	94.64±14.81^{ab}	38.50±8.37^{cd}	46.69±6.98^{e}
G	94.29±19.99^{ab}	36.49±4.54^{bd}	41.90±9.06^{e}
H	76.82±22.71^{b}	36.03±4.34^{bd}	45.34±16.72^{be}
I	58.74±8.07^{cdefg}	38.26±5.65^{d}	59.97±12.72^{e}
J	68.89±9.61^{fg}	34.91±4.68^{bd}	50.48±7.24^{e}
K	97.13±22.89^{abfij}	32.02±2.98^{bd}	50.57±9.13^{e}
L	84.15±5.75^{fgk}	30.67±3.08^{bd}	41.88±8.88^{be}

GSH (µmol/g tissue) NPT (µmol/mg protein) PT (µmol/mg protein)

L-NAME was given orally at 40mgkg^{-1}. Lisinopril was given at 0.28mgkg^{-1} and Extracts measured in mgkg^{-1}at the dosage of 100 (1), 200 (2) and 400 (3), respectively for the*Persea americana* leaf (PALE), bark (PABE) and root (PARE) extracts as given to groups B-K with L-NAME while A received distilled water and Lreceived L. NAME + Lisinopril.

Significance was measured at values presented as mean ± SEM. Groups. Significance was measured at $\alpha_{0.05}$, Means with superscript a - l showed significance when compared with their respective groups A-L.

Table 4.3.8: Antioxidant and modulatory effects of *Persea americana*leaf, bark and root extracts and lisinopril on the heart tissue of hypertensive rats.

	GLUTATHIOL-S-TRANSFERASE	SUPOXIDE DISMUTASE	GPX
A	0.37±0.21	16.92±4.20	163.4 ±3505
B	0.03 ± 0.01^{a}	1.94 ± 0.55^{a}	52.86 ± 3482^{a}
C	1.24 ± 0.30^{ab}	29.48 ± 5.74^{ab}	289.2 ± 33.03^{ab}
D	$1.86\pm0.29^{abc,}$	24.03 ± 5.74^{ab}	252.6 ± 16.38^{ab}
E	1.66 ± 0.31^{abc}	28.46 ± 2.95^{ab}	$291.341.54^{ab}$
F	0.09 ± 0.01^{cde}	16.03 ± 5.44^{bcde}	96.69 ± 21.57^{acde}
G	0.10 ± 0.02^{cde}	15.12 ± 6.01^{bcde}	95.77 ± 25.40^{acde}
H	$0.28\pm0.^{09cde}$	17.96 ± 3.72^{bcde}	109.8 ± 30.80^{abcde}
I	0.08 ± 0.01^{acde}	11.94 ± 2.52^{bcdeh}	102.81 ± 69.15^{abcde}
J	0.27 ± 0.15^{cde}	11.27 ± 1.03^{bcdeh}	98.33 ± 17.63^{acde}
K	0.55 ± 0.18^{bcdefgi}	10.67 ± 0.81^{abcdeh}	$223.25\pm17.69^{abcefghij}$
L	0.19 ± 0.16^{bcde}	16.47 ± 3.60^{bcde}	$237.71\pm17.06^{abcefghij}$

L-NAME was given orally at 40mgkg^{-1}. Lisinopril was given at 0.28mgkg^{-1} and Extracts measured in mgkg^{-1}at the dosage of 100 (1), 200 (2) and 400 (3), respectively for the*Persea americana* leaf (PALE), bark (PABE) and root (PARE) extracts as given to groups B-K with L-NAME while A received distilled water and Lreceived L. NAME + Lisinopril. GST (µmol-1-chloro-2,4-dinitrobenzene-GSH complex formed/min/mg protein), SOD (unit/mg protein), GPX (unit/mg protein)

Significance was measured at values presented as mean ± SEM. Groups. Significance was measured at$\alpha_{0.05}$, Means with superscript a - l showed significance when compared with their respective groups A-L.

.

Table 4.3.9: Antioxidant and modulatory effects of *Persea americana*leaf, bark and root extracts and lisinopril on the renal tissues of hypertensive rats.

GROUPS	GLUTATHIOL-S-TRANSFERASE	SUPOXIDE DISMUTASE	KIDNEY GPX
A	0.13±0.02	9.04±1.80	192.48±37.80
B	0.03±0.02	5.27±4.13	93.95±22.97^{a}
C	1.34±0.48^{ab}	7.81±2.34	237.87±7.79^{b}
D	1.55±0.84^{ab}	7.59±0.99	220.11±53.10^{b}
E	1.81±0.54^{ab}	8.98±1.10	269.6±4.227^{ab}
F	0.08±0.06^{cde}	10.06±8.40	112±52.97^{acde}
G	0.08±0.04^{cde}	16.19±0.85^{abcdef}	101.5±5.51^{acde}
H	0.06±0.04^{cde}	16.17±5.92^{abcdef}	111.5±27.46^{acde}
I	0.11±0.02^{cde}	7.36±1.62^{gh}	171.15±51.17^{bcefgh}
J	0.12±0.03^{cde}	9.59±1.05^{gh}	176.75±55.48^{bcefgh}
K	0.16±0.03^{cde}	9.59±0.99^{gh}	174.33±23.21^{bcefgh}
L	0.11±0.02^{cde}	7.98±1.07^{gh}	177.78±26.28^{bcefgh}

L-NAME was given orally at 40mgkg^{-1}. Lisinopril was given at 0.28mgkg^{-1} and Extracts measured in mgkg^{-1}at the dosage of 100 (1), 200 (2) and 400 (3), respectively for the*Persea americana* leaf (PALE), bark (PABE) and root (PARE) extracts as given to groups B-K with L-NAME while A received distilled water and Lreceived L. NAME + Lisinopril.GST (µmol-1-chloro-2,4-dinitrobenzene-GSH complex formed/min/mg protein), SOD (unit/mg protein), GPX (unit/mg protein).
Significance was measured at values presented as mean ± SEM. Groups. Significance was measured at $\alpha_{0.05}$, Means with superscript a - l showed significance when compared with their respective groups A-L.

Table 4.3.10: Serum myeloperoxidase and nitric oxide status in hypertension and the modulatory effect of *Persea americana*leaf, bark and root extracts and lisinopril

Groups	MPO	NO
A	98.13 ±19.13	0.71±0.27
B	15.63±9.13[a]	0.32±0.08
C	39.56±6.13[a]	1.08±0.85[b]
D	41.5±9.17[a]	1.90±0.83[bd]
E	51.05±4.20[ab]	1.03±0.64[bd]
F	53.43±15.24[ab]	0.46±0.09[d]
G	47.97±14.28[ab]	0.39±0.04[d]
H	65.36±18.54[ab]	0.42±0.15[d]
I	76.92±32.20[bc]	0.77±0.14[d]
J	94.38±29.26[bcdefg]	0.78±0.16[d]
K	93.37±26.51[bcdefg]	1.21±0.78[bfgh]
L	82.28±32.90[bcdefg]	0.80±0.21[d]

L-NAME was given orally at 40mgkg^{-1}. Lisinopril was given at 0.28mgkg^{-1} and Extracts measured in mgkg^{-1}at the dosage of 100 (1), 200 (2) and 400 (3), respectively for the*Persea americana* leaf (PALE), bark (PABE) and root (PARE) extracts as given to groups B-K with L-NAME while A received distilled water and Lreceived L. NAME + Lisinopril. MPO (µmol/L) NO ()

Significance was measured at values presented as mean ± SEM. Groups. Significance was measured at $\alpha_{0.05}$, Means with superscript a - l showed significance when compared with their respective groups A-L.

.

4.3.1 Immunohistochemistry of the Heart Tissues of L-NAME Induced Hypertensive Rats Treated with *P. americana* Extracts.

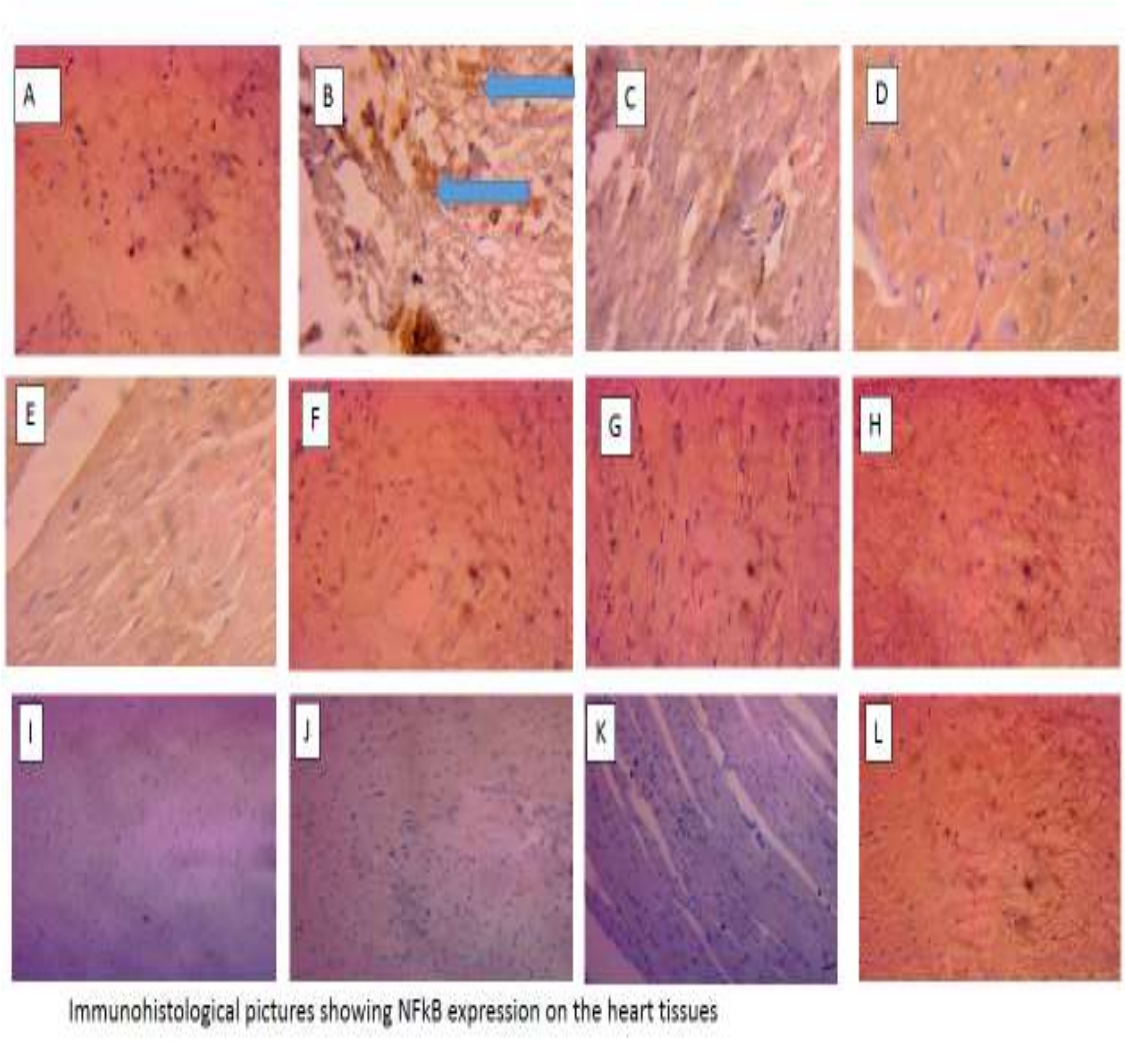


Figure 4.3.1 Photomicrographs of Immunohistochemical examination of heart sections of thehypertensive albino rats treated with *Persea americana*leaf, bark and root extracts and lisinopril using Nuclear Factor Kappa B primary antibody, at x100 magnification under a microscope. There was higher expression of NFkB in group B when compare with the control and every group treated with extracts of the leaf, bark and root and that given lisinopril.

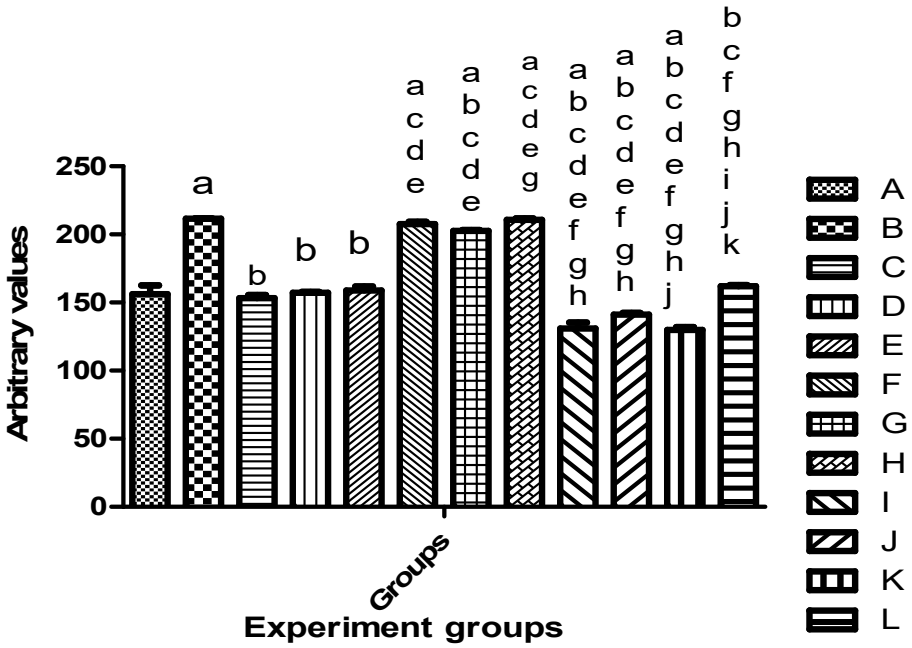


Figure 4.3.2 lmage J quantification of the photomicrogragh.of immunohistochemistry of NFkB protein expression in the cardiac tissues.There was significantly ($\alpha_{0.05}$) higher expression of NFkB in group B when compared with control and each of the groups treated with extracts of the leaf, bark and root and lisinopril.

4.3.2 Immunohistochemistry of the Heart and Kidney Tissues of L-NAME Induced Hypertensive Rats Treated with *P. americana* Extracts.

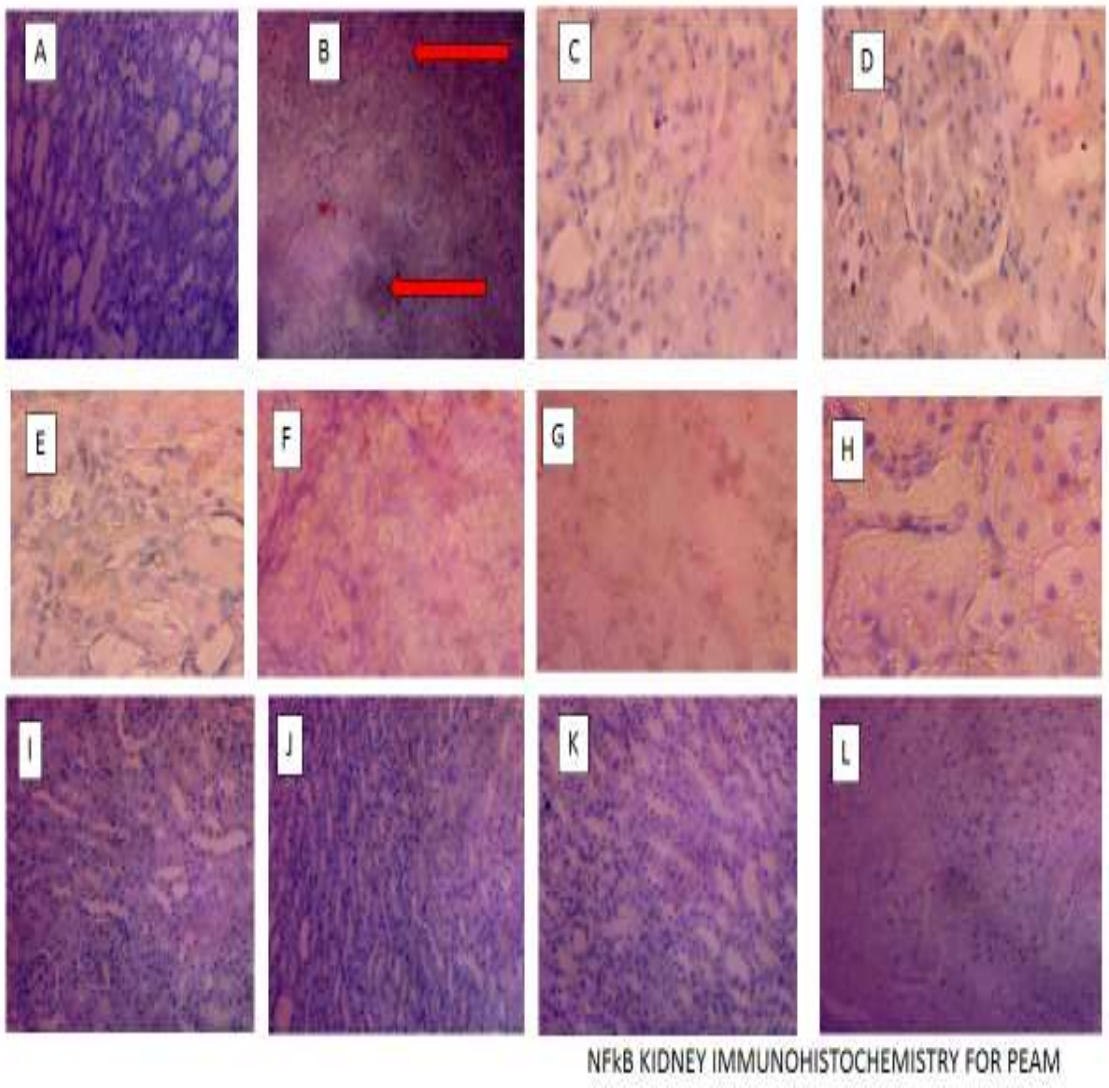


Figure 4.3.3 Photomicrographs of Immunohistochemical examination of heart and kidney sections of the hypertensve albino rats treated with *Persea americana*leaves, root and bark extracts and lisinopril using Nuclear Factor Kappa B primary antibody, at x100 magnification under a microscope. There was higher expression of NFkB in group B when compare with the control and each of the groups treated with extracts of the leaf, bark and root and that given lisinopril.

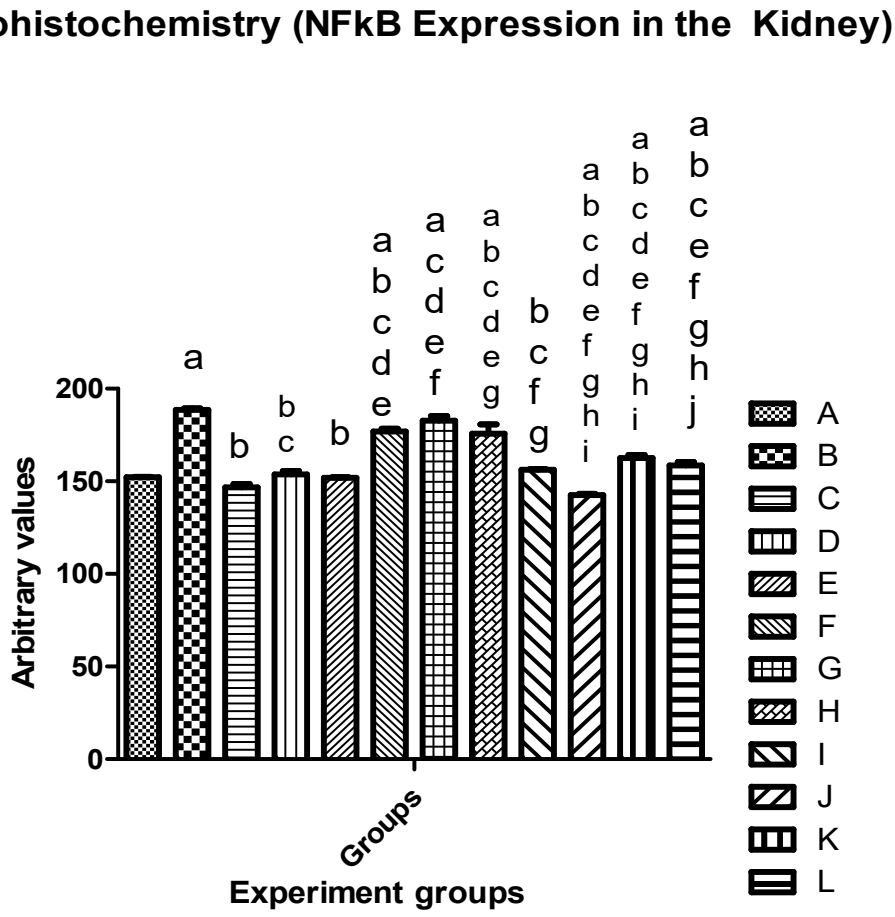


Figure 4.3.4 lmage J quantification of thephotomicrogragh.of immunohistochemistry of NFkB protein expressionin the kidney tissues. There was significantly ($\alpha_{0.05}$) higher expression of NFkB in group B when compared with control and the groups treated with extracts of the leaf, bark and root and lisinopril.

4.4Results of Phytcompounds isolation.

DPPH ASSAY OF THE SUBFRACTIONS OF ETHYL ACETATE FRACTION OF P. AMERICANA ROOT EXTRACT

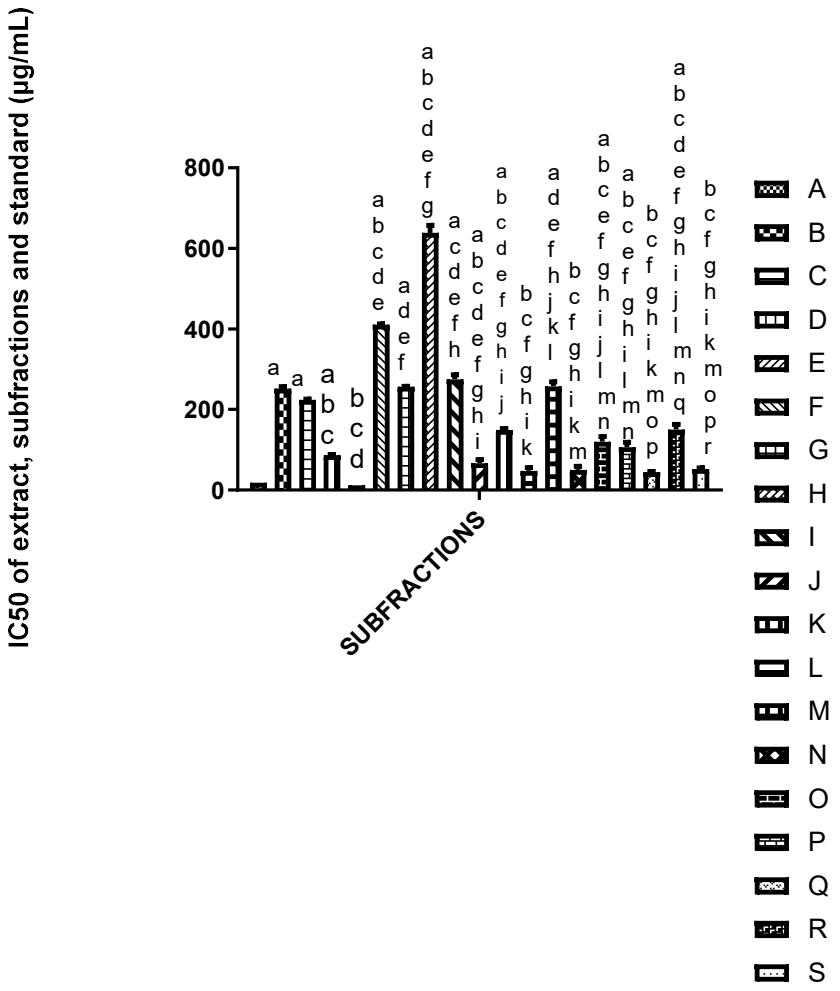


Figure 4.4.1: The IC 50 values of subfractions of the ethyl acetate fraction of the P.americana root in DPPH assay.Subfractions A-S. Superscripsts a - s. show that the groups were significant when compared with groups A - S respectively.subfraction

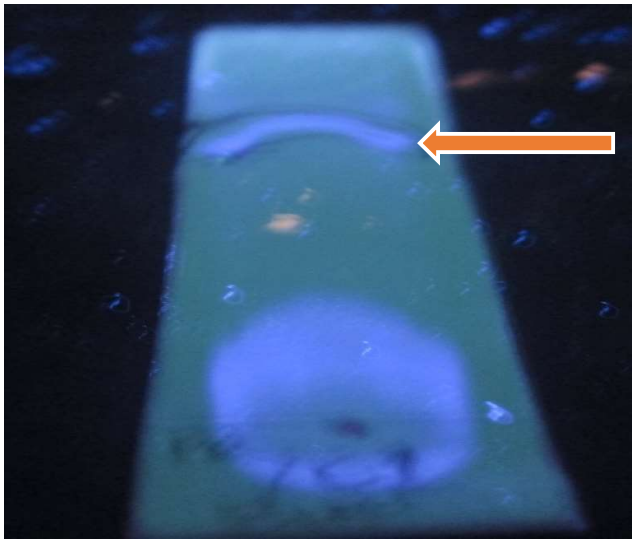


Figure 4.4.2 Pure isolated compound from the *P.americana* root butanol subfractions. (Viewed with UV lamp)

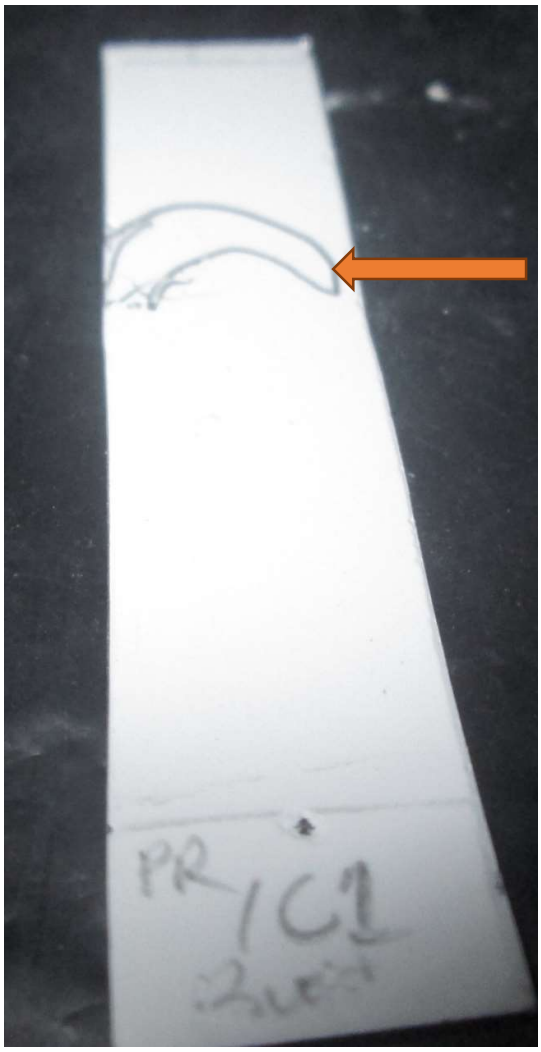


Figure 4.4.3 Pure isolated compound from the *P.americana* root butanol subfractions. (Viewed with UV lamp and marked with pencil)

Figure 4.4.4 Pure isolated compound from the *P.americana* leaf ethyl acetate subfractions. (Viewed with UV lamp)

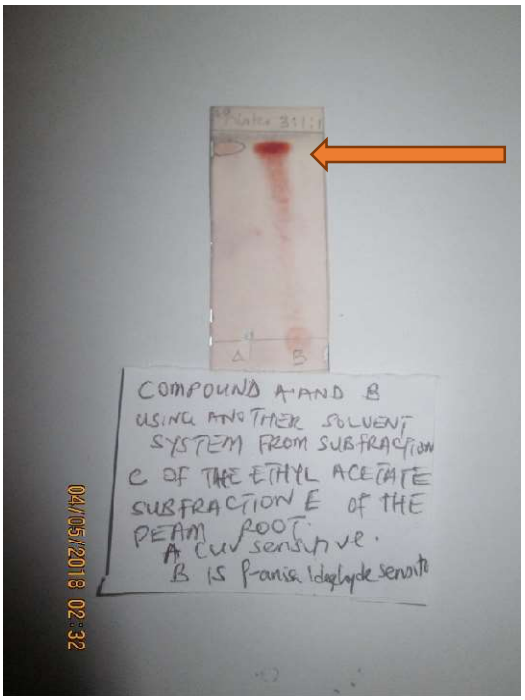


Figure 4.4.5Pure isolated compounds REACA and REACB from the *P.americana* leaf ethyl acetate subfractions. (Viewed with UV lamp)

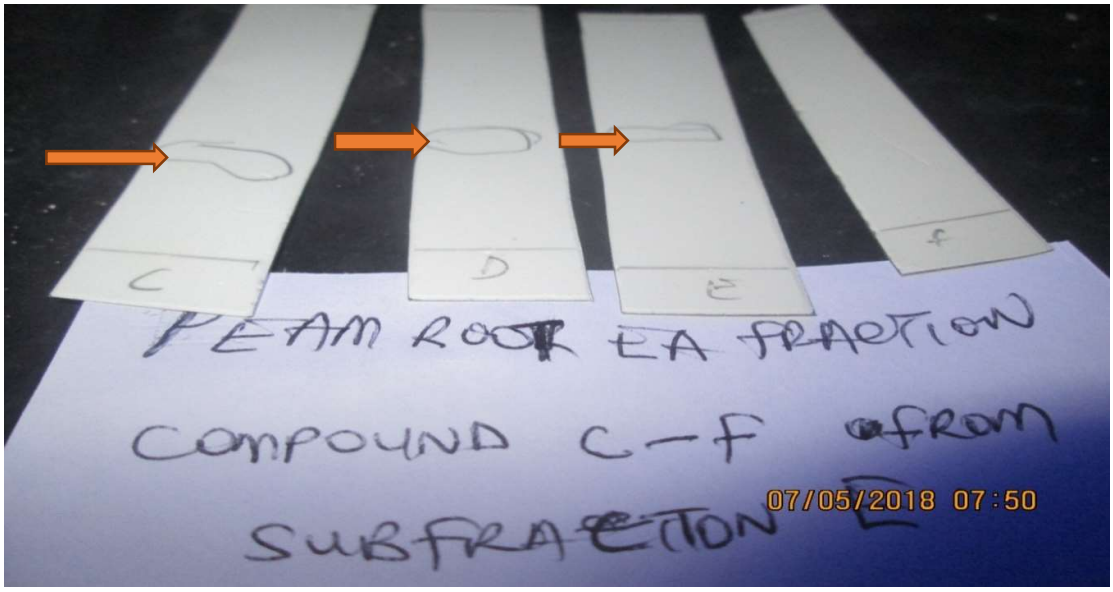


Figure 4.4.6Pure isolated compounds REACC, REACD, REACE and REACF from the *P.americana* root ethyl acetate subfractions. (Viewed with UV lamp and marked with pencil)

Table 4.4.1: The isolated pure compounds from the leaf and root extracts of *P. americana*

S/N	CODE	MEANING
1	RBCA	Root butanol compound A
2	REACA	Root ethyl acetate compound A
3	REACB	Root ethyl acetate compound B
4	REACC	Root ethyl acetate compound C
5	REACD	Root ethyl acetate compound D
6	REACE	Root ethyl acetate compound E
7	REACF	Root ethyl acetate compound F
8	LEACA	Leaf ethyl acetate compound A
9	LEACB	Leaf ethyl acetate compound B
10	LEACC	Leaf ethyl acetate compound C
11	LEACB	Leaf ethyl acetate compound D

4.5.0 Results of the In-vitro Antioxidant, Anti-inflammatory and Anti-hypertensive Assays of the Isolated Pure Compounds from the *Persea americana* Leaf and Root Extracts.

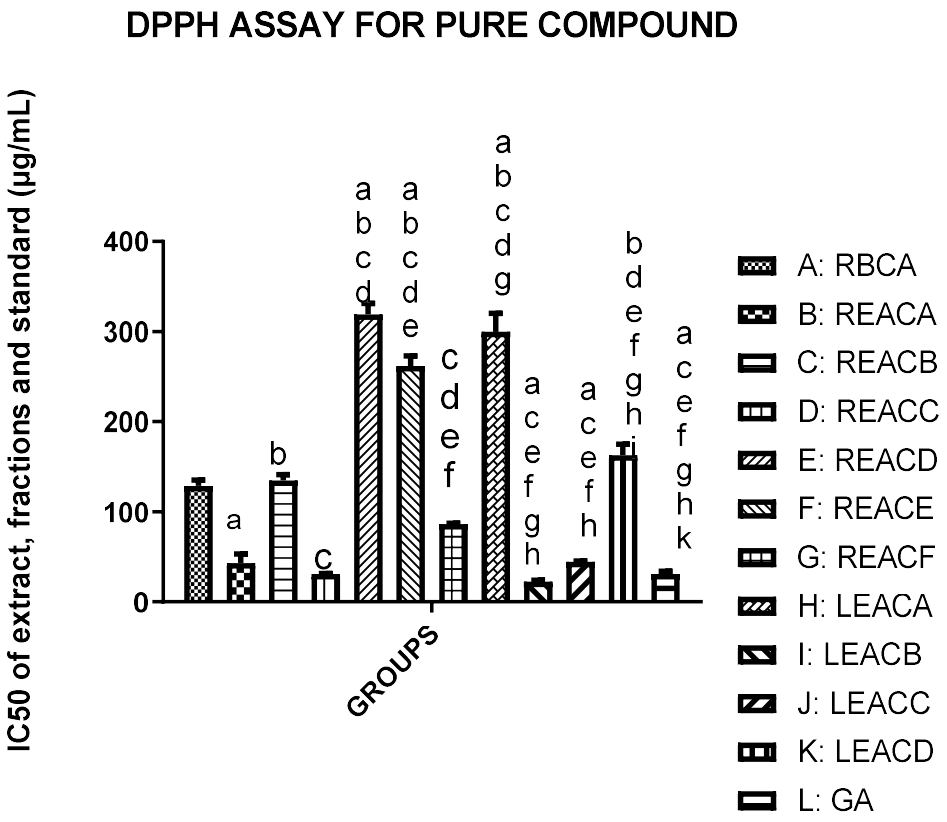


Figure 4.5.1: The result of DPPH assay of the pure compounds.

Group A: RBCA, Group B: REACA, Group C: REACB, Group D: REACC, Group E: REACD, Group F: REACE, Group G: REACF, Group H: LEACA Group I: LEACB, Group J: LEACC, Group K: LEACD, Group L: Gallic acid (GA). Superscripsts a,b,c,d, e, f, g, h, I, j, k and l show that the groups were significant when compared with their respective groups A-L. Significance at $\alpha_{0.05}$

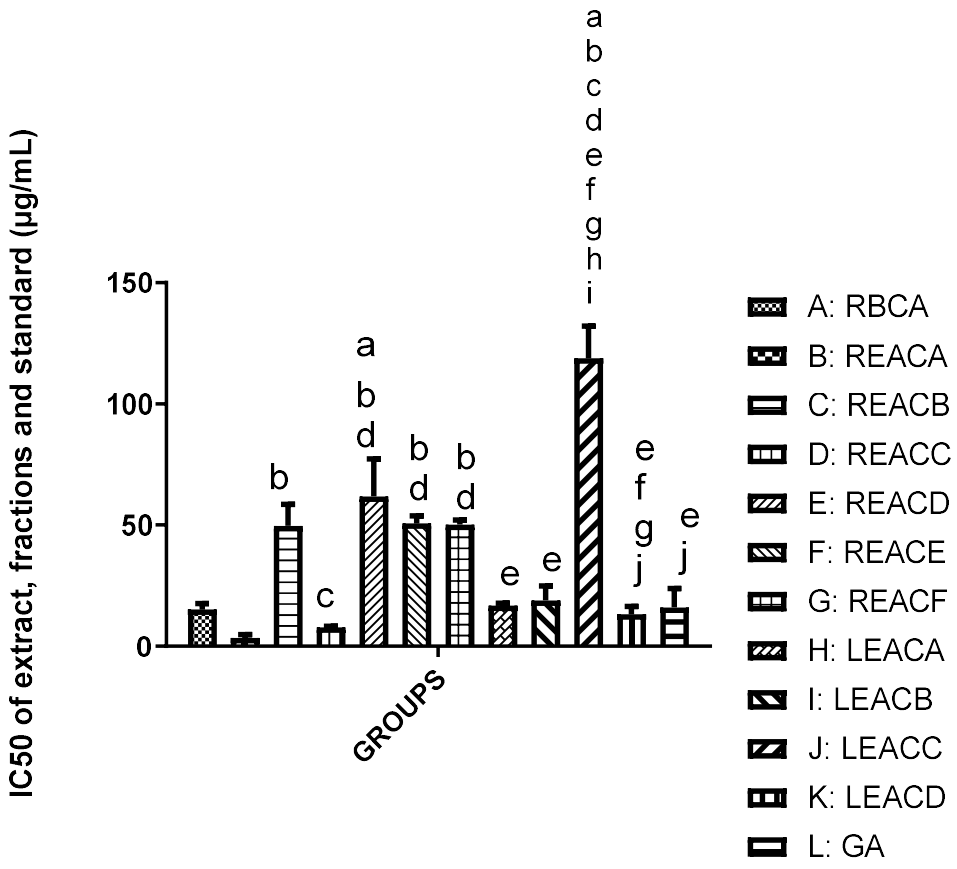


Figure 4.5.2The result of ABTS assay of the pure compounds.

Group A: RBCA, Group B: REACA, Group C: REACB, Group D: REACC, Group E: REACD, Group F: REACE, Group G: REACF, Group H: LEACA Group I: LEACB, Group J: LEACC, Group K: LEACD, Group L: Gallic acid (GA). Superscripsts a,b,c,d, e, f, g, h, I, j, k and l show that the groups were significant when compared with their respective groups A-L. Significance at $\alpha_{0.05}$

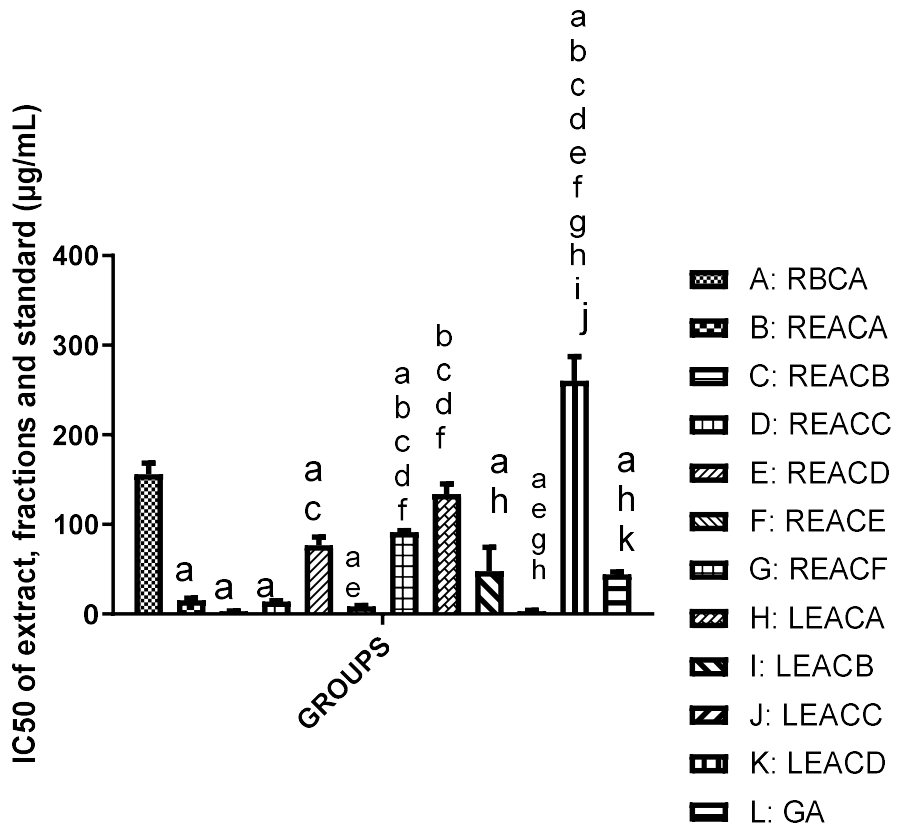


Figure 4.5.3: The result of Hydroxyl radial scavenging assay of the pure compounds.

Group A: RBCA, Group B: REACA, Group C: REACB, Group D: REACC, Group E: REACD, Group F: REACE, Group G: REACF, Group H: LEACA Group I: LEACB, Group J: LEACC, Group K: LEACD, Group L: Gallic acid (GA). Superscripsts a,b,c,d, e, f, g, h, I, j, k and l show that the groups were significant when compared with their respective groups A-L.Significance at $\alpha_{0.05}$

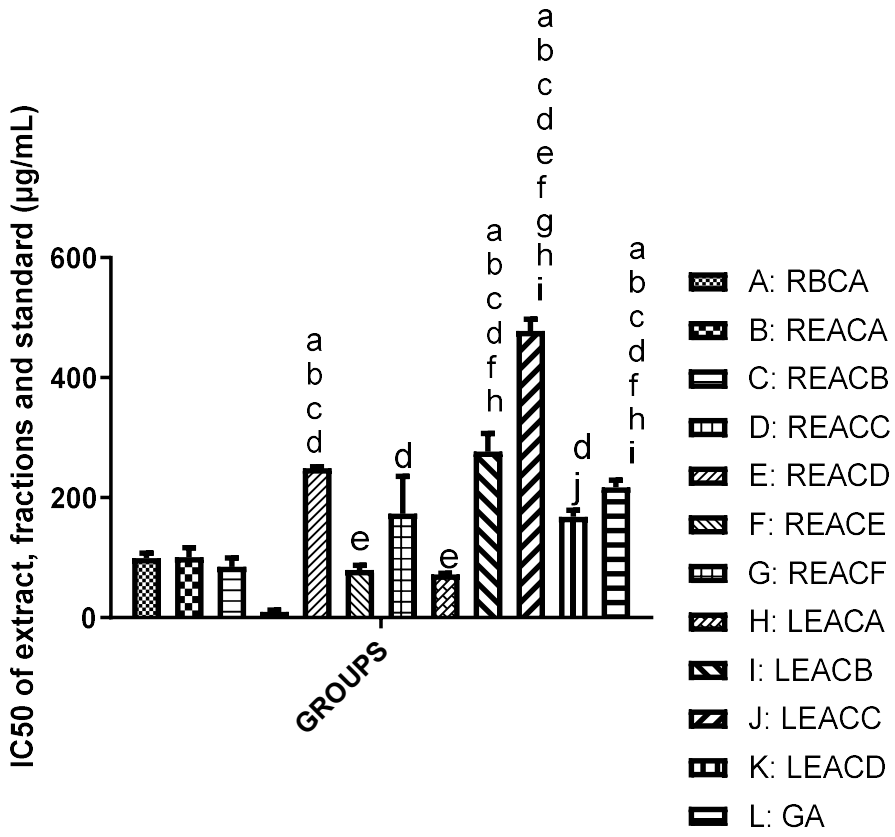


Figure 4.5.4: The result of Metal chelating assay of the pure compounds.

Group A: RBCA, Group B: REACA, Group C: REACB, Group D: REACC, Group E: REACD, Group F: REACE, Group G: REACF, Group H: LEACA Group I: LEACB, Group J: LEACC, Group K: LEACD, Group L: Gallic acid (GA). Superscripsts a,b,c,d, e, f, g, h, I, j, k and l show that the with their respective groups A-L. Significance at $\alpha_{0.05}$

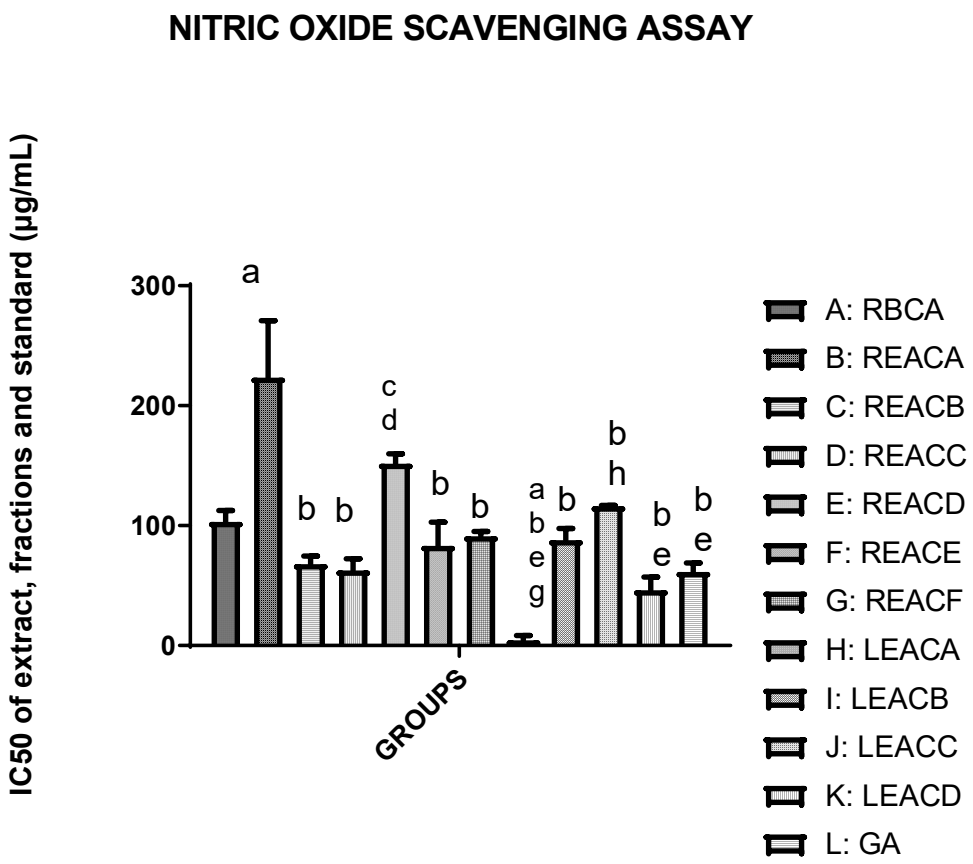


Figure 4.5.5The result of Nitric oxide assay of the pure compounds.

Group A: RBCA, Group B: REACA, Group C: REACB, Group D: REACC, Group E: REACD, Group F: REACE, Group G: REACF, Group H: LEACA Group I: LEACB, Group J: LEACC, Group K: LEACD, Group L: Gallic acid (GA). Superscripsts a,b,c,d, e, f, g, h, I, j, k and l show that the groups were significant when comparedwith their respective groups A-L. Significance at $\alpha_{0.05}$

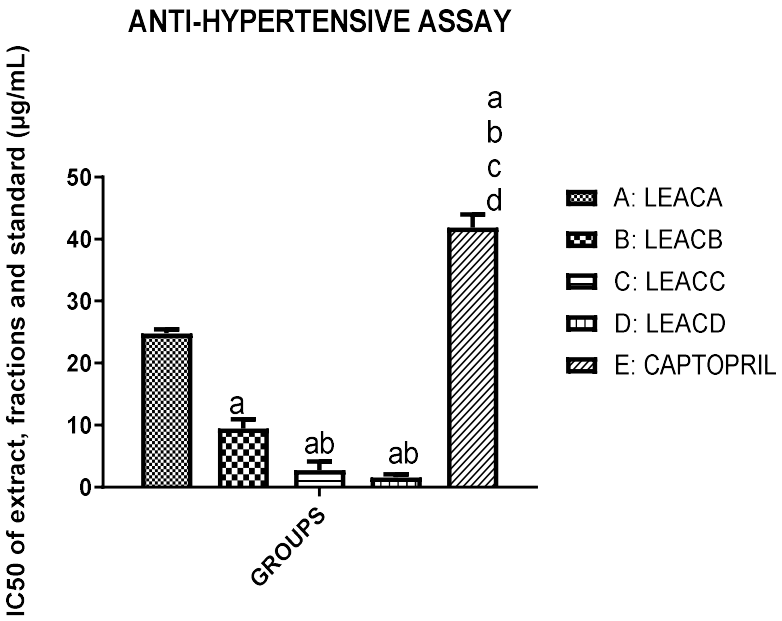


Figure 4.5.6:Anti-hypertensive assay of the pure compounds from the leaf extracts.

Group A: LEACA, Group B: LEACB, Group C: LEACC, Group D: LEACD, E: Captopril. Superscripsts a, b, c, d, and e show that the groups were significant relative to groups A- E, respectively. Significance at $\alpha_{0.05.}$

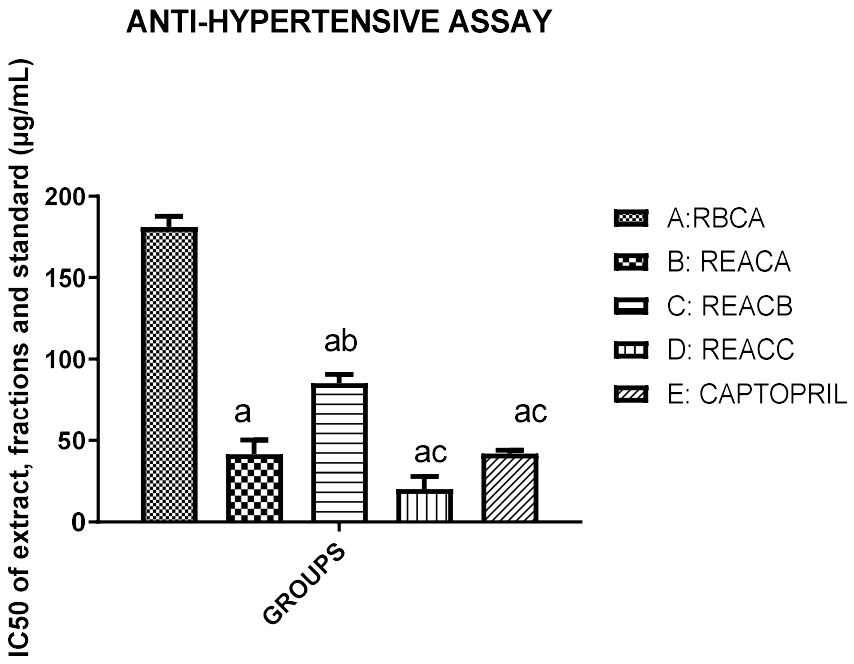


Figure 4.5.7: The result of anti- hypertensive assay of the pure compounds from the extracts of *P. americana*root.

Group A: LEACA, Group B: LEACB, Group C: LEACC, Group D: LEACD, E: Captopril. Superscripsts a, b, c, d, and e show that the groups were significant relative to groups A- E, respectively.. Significance at $\alpha_{0.05.}$

CHAPTER FIVE

DISCUSSION

5.1*In vitro* Antioxidant, Anti-inflammatory and Anti-hypertensive Assays on *P.americana* Leaf, Bark and Root Extracts and Fractions.

Phytochemicals constituents like alkaloids, glycosides, flavonoids, phenols, saponin, triterpenes and phytosterols in plants have been reported to be responsible for the biological activities of medicinal plants (Alimi and Ashafa, 2017). *P. americana*leaf, bark and root extracts possess all these phytochemicals and this justify the pharmacological effects of the extracts used in this work. Phenols have been reported to have free radical scavenging and cellular damage modulatory effects (Herrara *et al*, 2009). Alkaloid of plants are well documented to have free radical scavenging, cardiovascular and organ damage modulatory effects. (Akinmoladun *et al*, 2007). Flavonoids have been documented to have stong free radical scarveging, anti-cancer and anti-inflammatory activities (Okwu 2004). Phytosterols have been reported to have lymphoproliferative and immunomodulatory effects (Bouje 2001).

The anti-oxidant and free radical scavenging actions of medicinal plants is majorly dependent on their high poly-phenolic rich compounds and flavonoids (Huang, 2010, Maisuthisukul *et al*., 2012).

Flavonoids in Avocado leaf extract is a good anti-oxidants that scavenge free radicals by donating hydrogen atoms or by single electron transfer (Nurdian *et al.*, 2018). *P. americana* leaf extracts contains all the beneficial phytochemicals including phenols, flavonoid, alkanoids, phytosterol and these explain the free radical scavenging activities of the *P.americana* leaf, bark and root extracts and isolated phytocompounds. *P. americana* plant extracts possess strong free radical scavenging activities like metal chelating, iron reducing power, hydroxyl radicals, DPPH radical and ABTS radical scavenging activities and can modulate diseases and organ damage

cause by oxidative stress. The plant extracts are potent and can therefore be used in the management of hypertension.

P. americana leaf extract and fractions had variable free radical scavenging activities with variable potency as shown by the IC 50 values.

*P. americana*Leaf Extract

The results of these studies showed that the leaf extract and fractions had good antioxidant, anti-inflammatory and anti-hypertensive properties. The crude methanol extract of the leaf had significantly lower IC50 value and better metal chelating activities when compared with the garlic acid (group G). Also the N-hexane, Ethyl acetate, N-Butanol and aqueous methanol fractions had lower and comparable metal chelating activity with that of gallic acid a standard antioxidant. The metal chelating activity of chloroform fraction of *P. americana* leaf extract had a lesser metal chelating activity when compared with gallic acid.

The ABTS assay showed that the crude methanol leaf extract, N-hexane fraction, Ethyl acetate fraction, and N-Butanol fraction had ABTS radical scavenging activities that were better than that of gallic acid. N-Butanol fraction gave the best ABTS scavenging activity. The chloroform and aqueous methanol fraction of the *P. americana* leaf has better hydroxyl radical scavenging activities when compared with garlic acid, a standard antioxidant. Crude methanol extract and ethyl acetate also had hydroxyl savaging activity although their activities were not as potent as gallic acid. The difference in potency however was not statistically significant.

The DPPH radical scavenging assay result showed that the crude methanol extract of the leaf, N-hexane and ethyl acetate factions had DPPH radical scavenging activities comparable to that of gallic acid. However, the DPPH scavenging activity of aqueous methanol extract was the best of them as the IC50 was significantly lower than those of the crude methanol extract, other fractions and the standard (GA).

The nitric oxide radical of the leaf revealed that the crude methanol extract, N-hexane fraction and ethyl acetate fraction had more potency in nitric oxide scavenging activities than that of garlic acid. The chloroform fraction of the leaf had comparable nitric oxide scavenging activity with gallic acid.

The reducing power assay revealed a dose dependent reducing power ability of ethyl acetate and n-butanol fractions were comparable to that of garlic acid as from 62.5-

250µg/ml concentration of the drugs while that of aqueous methanol was comparable to that of GA at the concentration of 250 µg/ml.

It was also shown from the inflammatory assay that n-hexane, chloroform, ethyl acetate, N-butanol and aqueous methanol had better anti-inflammatory activity than indomethane, a standard anti-inflammatory drug.

Crude methanol extract of *P. americana* leaf was not as potent as captopril. It is required at the concentration of 250 µg/ml used. It requires higher concentration of crude extract to perform the same effect as captopril.

*P. americana*Bark Extract

Furthermore, *P. americana* bark extract and fractions had variable free radical scavenging activities with variable potency as shown by the IC 50 values.

The crude methanol bark extract, n-butanol and aqueous methanol fractions were more potent than the gallic acid in metal chelating assay. The ethyl acetate fraction had a comparable activity as gallic acid. This showed that the crude methanol and aqueous methanol have potent metal chelating activity that was stronger than that of the gallic acid. Ethyl acetate fraction was equal in metal chelating ability as gallic acid. Hence, the crude bark extract, ethyl acetate, n-butanol and aqueous methanol fractions have ability to reduce oxidative stress through free radicals scavenging.

In DPPH radical scavenging assay of the bark of *P.americana*, the crude methanol extract, chloroform and ethyl acetate fractions had DPPH radical scavenging activities that is comparable to that of the gallic acid, a standard antioxidant. This showed that they are capable of reducing or ameliorating oxidative stress induced pathology in animals.

In the ABTS assay of the *P. americana* bark, the crude methanol had a very strong ABTS radical scavenging ability comparable to that of ascorbic acid, a standard anti-oxidant. The other fractions were not as potent as ascorbic acid in ABTS radical scavenging ability.

The IC50 value of the extracts and fractions in nitric oxide scavenging assay showed that n-hexane and n-butanol fractions had comparable nitric oxide scavenging ability. The crude methanol extract, the ethyl acetate and aqueous methanol fractions were lower in potency when compared with gallic acid.

In hydroxyl radical scavenging assay, the aqueous methanol fraction had a very potent hydroxyl radical scavenging activity. The crude methanol extract had relatively equal hydroxyl radical activity to that of n-hexane and chloroform fractions.
Ethyl acetate and n-butanol had very poor hydroxyl radical scavenging ability.
A dose dependant reducing power activity was observed crude methanol extract, chloroform and, ethyl acetate fractions at 125µg/ml and 250µg/ml in these effects were comparable to those of gallic acid at 62.5 µg/ml and 125µg/ml.

In anti-inflammatory assay the ethyl acetate fraction had more potent anti-inflammatory activity than quercetin. Similarly, antiflammatory activities of the crude methanol bark extract, n-butanol, chloroform and ethyl acetate fractions were better than that of indomethacin in this study.

Anti-hypertensive assay showed that the crude methanol bark extract had better anti hypertensive activity than that of captopril. It is therefore more potent than captopril in ACE inhibition and can be used for effective management of hypertension.

P. americana Root Extract
In vitro assay of the *P.americana* root extracts revealed that the metal chelating activity of the root extract, N-Hexane, chloroform and n-butanol fractions were more potent than gallic acid.
The crude methanol extract, ethyl acetate and aqueous methanol fraction had comparable metal chelating activity.
ABTS radicals scavenging assay of the *P. americana*root extract showed that the crude methanol extract, n-butanol and aqueous methanol fraction were more potent in ABTS radical scavenging activities than gallic acid.

DPPH scavenging activity of the crude methanol root extract is similar to that of gallic acid. N-butanol and aqueous methanol fraction had more potent activities on DPPH scavenging than gallic acid. Chloroform fraction was more potent than gallic acid with a significantly higher IC50 than that of gallic acid.
In hydroxyl radical scavenging assay, the crude methanol root extract was not as potent as gallic acid in hydroxyl radical scavenging activity. The n-hexane fraction was the most potent of the fractions and gallic acid. The chloroform, ethyl acetate, N-butanol and aqueous methanol had low hydroxyl radical scavenging activity.

The reducing ferric antioxidant power of *P. americana* root was dose dependent. The chloroform, ethyl acetate, aqueous methanol fractions had reducing power that was similar to that of gallic acid. The reducing power antioxidant assay of chloroform fraction, ethyl acetate fraction and gallic acid at the concentration of 125µg/ml and 250µg/ml and aqueous methanol fraction at the concentration of 250µg/ml were greater than that of the crude methanol at the same dose.

The anti inflammatory activity of the ethyl acetate was comparable to that of Quercetin. Crude methanol extract, n-hexane, chloroform, n-butanol and aqueous methanol fractions showed better anti-inflammatory activities when compared with that of indomethacin, a standard anti-inflammatory drug.

The mechanisms of action of many phenolic compounds such as flavonoids, tannins and curcumins were via their free radical scavenging activities or the inhibition of pro-inflammatory enzymes such as cyclooxygenases (COX) and lipoxygenases (LOX) in the inflammatory cascades (Sadik *et al.,* 2003 and Lee *et al.,* 2003). Flavonoids were reported to inhibit prostaglandins, end product in the COX and LOX pathways of immunologic responses (Nilvetidis *et al.,* 2001).There are COX 1, COX 2 and COX 3 which are selectively inhibited by acetaminophen and related compounds (Chandraselcharan *et al.,* 2002 and Botting, 2003).

The selective inhibition of COX-2 is more desirable because the inhibition of COX 1 in the gastric mucosa causes gastric ulceration and bleeding that is associated with the undesirable effect of non-steroidal anti-inflammatory drugs (NSAIDS)(Bezakova *et al.,* 2007). COX-2 is induced as an early response to pro-inflammatory mediator and stimuli such as endotoxins and cytokines (Chadea and Lisaka, 2005). Induced COX-2 synthesizes prostaglandins that contribute to inflammation swelling and pain. Compounds that effectively inhibit COX-2/LOX can effectively inhibit the biosynthesis of prostaglandins and leukotrienes from arachidonic acid and can therefore be used in the treatment of inflammation (Brand-Williams,*et al.,* 1995 and De Wet, 2011) without the undesirable effect that is associated with NSAIDS.

The anti-hypertensive assay revealed that the crude methanol extract was not as potent as captopril, although the difference in IC50 was not significant. Higher concentration of the extract will be needed to have similar effect as captopril.

5.2. Acute Toxicity Study

5.2.1 *P. americana* leaf extract

The acute toxicity study of the leaves extract showed that there was no significant effect on the systolic (SBP), diastolic (DBP) and mean arterial pressure (MAP) of the rats acutely dosed with the leaves extract. However, slight decreases were observed across the groups acutely dosed with the extract relative to the control group.This shows a hypotensive effect of the extract in normotensive rats as reported by Ojewole *et al*. (2007).

The extract had no significant effect on the haematology (PCV, Hb, RBC, WBC, neutrophils, lymphocytes, monocytes and platelet count, MCV, MCH and MCHC) of the rats across all groups. This finding agrees with that of Ozolua *et al*. (2009), who found no effect on the blood cell indices of experimental rats after a maximum dosage of 10mg/kg with aqueous extract of *P. americana* for 28days.

5.2.2 P. americana bark extract

Acute toxicity study of the methanol extract of the bark revealed a dose dependent percentage increase in the body weight of the rats across the group which does not agree with Brai *et al.*(2006)that found catabolic activity of the aqueous bark extract of *P. americana* on the stored lipids in the adipose tissue.

Statistically non-significant effects were observed in the systolic, diastolic and mean arterial pressure (MAP) of the rats dosed with the extract. However there was a slight decreaseacross the groups dosed with the extract compared to the control group A. This shows a hypotensive effect of the extract in normotensive rats as reported by Ojewole *et al.,*(2007).

There was a non-statistically significant effect of the extract on the haematological parameters (PCV, Hb, RBC, WBC, Neutrophil, Lymphocytes, Monocyte, and Eosinophil) of the rats across all groups. This finding is in agreement withOzolua *et al.,* (2009) that reported that there was no effect on the haematological parameters of experimental rats after a maximum dosage of 10mg/kg with aqueous extracts of bark of *P.americana*for 28days.

5.2.3*P. americana* root extract

Acute toxicity study of the root extract showed a non-significant decrease in the packed cell volume and haemoglobin levels of group B, C, D compared to the control group. This signifies anaemia at the dosages 500 mg/kg, 1000 mg/kg respectively.

Serum transaminases are considered as sensitive indicator of hepatic damage. As a result of the injury to the liver, the cell membrane permeability and transport function are impaired which leads to a rise in the level of transaminases in the serum (Mahalaxmi *et al.,* 2011). The current results shows that there was a non-significant increase of creatinine, AST, ALP levels in the group given 7500mg/kg compared to the control group. This indicated renal and hepatic impairment at the dose of 7500mg/kg.

5.2.4 Total Phenolic Contents of the Leaf, Bark and Root.

The total phenolic content (TPC) result of the leaf extract showed that aqueous methanol fraction had the highest concentration of phenolic compound while N-butanol fraction was next to it. The crude methanol extract and ethyl acetate fraction had about equal content of phenolic compounds. N-hexane and chloroform fractions had the least TPC.

The TPC assay of the bark extract and fraction also revealed that the chloroform and ethyl acetate fractions had the highest phenolic content, followed by the N-hexane fraction.

The TPC assay of the root showed that the phenolic content of aqueous methanol fraction and crude methanol extract were the highest while that of the n-hexane fraction had the lowest content.

5.2.5 Qualitative Phytochemical Analysis of the Root Extract.

Phytochemical analysis of the root extract revealed that the root extract contains phenols, saponins, triterpines, flavonoids, alkaloids and phytosterol. This is similar to the findings by Nurdin *et al*. (2017) who found flavonoids, saponins, polyphenols, tannins, alkaloids and steroids in leaf and Temitope *et al*. (2017) who found from their phytochemical analysis of the bark, the presence of phenols, saponins, reducing sugar, steroids, tannins, flavonoids, alkaloids and cardiac glycosides.Tannin is a contistuent

of the leaf and bark that is absent in the root. This may be attributed to the environment or exposure of the leaf and back to sunlight which the root does not enjoy.

5.3 THE THERAPEUTIC EFFECT OF THE METHANOL LEAF, BARK AND ROOT EXTRACTS OF PERSEA AMERICANA ON L-NAME INDUCED HYPERTENSION IN WISTAR RATS.

The antihypertensive activity of *P. americana* extracts in hypertensive rats was explored in this study.

The rise in Systolic, Diastolic and MAP of Group B (Hypertensive rats) beyond the normal limit indicated hypertension which is related to the reportofGardiner *et al.,* (1990a), Fozard and Part, (1991). *P.americana* leaf caused a reduction in the blood pressure of rats treated with the extract. The decrease in the systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial blood pressure (MAP) of the hypertensive rats treated with *P. americana* leaf extracts compared with the hypertensive untreated rats(Table 4.3.2) showed a responsive treatment of the extracts on hypertension indicating anti-hypertensive effect as found by Owolabi *et al*. (2010) and Ojewole *et al*. (2007). There was no significant ($\alpha_{0.05}$) difference in the SBP, DBP and MAP of the groups treated with extracts and that treated with lisinopril therefore showing an averagely equal therapeutic antihypertensive potency of the extract compared to lisinopril, a standard ACE inhibitor. *Persea americana*bark extract significantly lowered the systolic, diastolic and mean arterial blood pressure values and corrected the resultant cardiac arrhythmia in this study to values comparable to lisinopril, an ACE inhibitor.

Oral medication with the aqueous methanol bark extract caused a remarkable reduction in the blood pressures of hypertensive rats with the best outcome at the dose level of 200 mg/kg of extract. The findings showed that the avocado bark extract had anti-hypertension effects in hypertensive rats models. The anti-hypertensive effects of avocado pulp has been reported by Takano (2009). The anti-hypertensive effect of avocado has been linked to their high constituent of potassium (Takano, 2009). Potassium causes blood vessels dilation and regulation of blood pressure and sodium

concentration. If taken adequately, potassium can remarkably reduce the risk of cardiovascular diseases incidence (Wood, 1988).

In this study, L-NAME administration for a period of 21 days caused hypertension as revealed by the remarkable increase in SBP, DBP and MAP values. L-NAME is known to cause inhibition of endothelial nitric oxide synthase (eNOS) thereby reducing the bioavailability of nitric oxide, a potent vasodilator in the vascular endothelium (McCarthy *et al*. 2011). *P. americana* is known to contain rich source of bioactive phytochemicals; including phenolic acids, condensed tannins, and flavonoids associated with multiple regenerative effects (Dias *et al*., 2016). In addition, *P.americana* has antioxidant potential for the treatment of numerous diseases (Castro-López *et al*., 2019). The reduced bioavailability of NO is associated with hypertension and vascular endothelium injury (Todorich *et al*., 2011). Studies have demonstrated that flavonoids and antioxidant properties in *P.americana*were able to improve vascular endothelium by increasing the bioavailability of NO and also attenuate hypertension (Rodríguez-Carpena *et al*., 2011; Bost *et al*., 2013). Moreover, the oxidative stress resulting from reduced bioavailability of NO appears to induce the release of several factors, including inflammatory cytokines and antiangiogenic factors, which culminate with endothelial dysfunction (Poniedziałek-Czajkowska *et al.*, 2011).

Several studies have elucidated the importance of NO in the pathogenesis of hypertension, it plays a major role in the regulation of blood pressure and the impairment of nitric oxide bioavailability is an important part of hypertension (Hermann *et al*., 2006). Some antihypertensive like sodium nitroprusside act by exploiting the effect of NO on vascular smooth muscle, they elicit the release of nitric oxide and thus facilitates vasodilation (Adefegha and Oboh 2016). In this study, we reported significant decrease in NO bioavailability in the hypertensive untreated rats whereas the groups treated with *Persea americana*and Lisinopril exhibited significant increases in NO bioavailability. Thus, suggesting that *Persea americana*leaf, bark and root extracts act by enhancing nitric oxide bioavailability.

In this present study, we reported significant elevations in various markers of oxidative stress such as malondialdehyde, protein carbonyl, hydrogen peroxide in the renal and cardiac of the L-NAME alissues of the hypertensiveuntreated one group.

Treatment with *Persea americana*leaf, back and root extracts however brought their values to levels comparable with the control and lisinopril treated groups.

Oxidative stress which is defined as an imbalance in the levels of oxidants and antioxidants favoring oxidants has been implicated in the advent of hypertension. It causes damage to macromolecules such as DNA, protein and lipids and it is exacerbated by insufficient antioxidants in the body (Wu and Harrison, 2015). Renal and cardiac dysfunction in hypertension is associated with oxidative damage (Ref).

In the kidney, oxidative stress promotes vasoconstriction, increases sodium retention and increases vascular resistance, therefore worsening hypertension (Duni *et al*. 2018). Cardiac diseases is also associated with oxidative stress (Jin *et al*. 2017).

Several antioxidants in the body act as the first line of defence against the damaging effect of free radicals and reactive oxygen species. These anti-oxidants include superoxide dismutase, reduced glutathione and glutathione peroxidase. (Nguyen et al., 2009). It is an established fact that consuming food with high concentrations of these antioxidants is valuable in prophylactic and therapeutic management of various cardiovascular and renal diseases (Smith *et al*. 2016). SOD acts by removing superoxide radical thus preventing the deleterious effects of superoxide radical (Kabel 2014). The glutathione system including reduced glutathione, glutathione peroxidase, glutathione transferase functions in detoxification metabolism. Reduced glutathione functions in sustaining the redox system of the cell (Kabel 2014). In this study, L-NAME significantly elicited a depletion in levels of these antioxidants in the renal and cardiac homogenates, however, treatment with *P.americana* and lisinopril caused significant increases in their levels, confirming the antioxidant effects of *P. americana*.

The decrease obtained in the renal and cardiac antioxidant enzymes showed that the production of ROS in hypertensive rats was overwhelming over the antioxidant capability thereby causing oxidative stress. Chronic hypertension led to reduction in cardiac and renal SOD, GST and GPx of rats. In addition, there was reduction in non-protein thiol (NPSH) and total thiol in the cardiac and renal tissues. The GSH is an essential antioxidant defence molecule within the cell and it is also serves as a substrate the activites of GPx and GST in metabolic detoxification, breaking down

and removal of toxic products in the body. Cardiac and renal antioxidant enzymes (SOD, GST, GPx, and CAT) quantity, however, were increased in hypertensive rats co-treated with *P.americana* leaf, bark and root extracts. These findings are linked to the antioxidant and flavonoids properties in the plant extracts.

In hypertensive rats aremarkable elevation of indicators of oxidative stress in the heart and kidney tissues of the rats as shown by the level of MDA and H_2O_2. For the groups co-administered with *P.americana*, the antioxidant and flavonoids content were capable of attenuating the oxidative stress level and concomitant recovery was observed.

MPO is a marker of inflammation and it catalyses the reactions that produce radicals like hypochloride and NO bioavailability (Abu-Soud *et al*., 2000).Inflammation is associated with oxidative stress, and both act in an interrelated way in hypertension (Duni *et al.* 2018). In this study, myeloperoxidase, a marker of inflammation was significantly elevated in the hypertensive untreated group confirming the role of inflammation in the pathogenesis of hypertension, *Persea americana*leaf, bark and root extracts however significantly depleted its levels in the treated groups.

Histological examination, in this this study, revealed variable distruptions in the heart and kidney tissues of hypertensive rats. Chronic hypertension revealed permeation of the myocardium by cells of inflammation and fats in the heart and focal areas of renal cast and interstitial infiltration by inflammatory cells in the kidney (Masuyama *et al*., 2000). Additionally, mild congestion of vessels and focal area of renal casts and loss of of the tissues in rats treated with L-NAME for a period of 21 days. With co-administration with *P.americana*leaf, bark and root extracts, pathology was attenuated in cardiac and kidney tissues in such a pattern that is directly related to the dose of the extract.

The immunohistochemistry of this study, revealed that hypertension led to the increased expression and regulation of NFκB protein increase, but significantly,*P. americana*leaf, bark and root extracts suppressed and inhibited inflammation because lesser ROS are available to activate the transcription factors NF-kB induce proinflammatory cytokines (MacNee, 2001).

NFkB modulates cell growth, cell survival, development processes, immunological processes, inflammation and apoptosis and its activation has been linked to a number of cancers. Its role in oncogenesis was attributed to the trigger of the activation of reactive oxygen species (Kusano and Ferrari 2011), inflammatory signals from the tissue also serves as an activator of NFkB and it has been implicated in the pathogenesis of several diseases including hypertension, cardiomyopathy and diabetes (Kusano and Ferrari 2011). Its down regulation in the renal and cardiac tissues of the extracts treated groups in this study, suggested that *P. americana*leaf, bark and root extracts possessactivities against inflammation and cell death.

5.4 Isolation of pure Metabolites.

Elevenisolated pure bioactive metaboliteswere from butanol fraction of the root and ethyl acetate fractions of the leaf and root. The isolation was guided by the anti-oxidant activities of the fractions. These metabolites were coded; RBCA, LEACA, LEACB, LEACE, LEACD, REACA, REACB, REACC, REACD, REACE, and REACF.

5.5Biological Assay for Pure Metabolites.

DPPH assay showed that LEACB and REACC had DPPH radical scavenging activities that is better than that of garlic acid while REACA. REACF and LEACE had similar DPPH radical scavenging activities to that of garlic acid. RBCA, REACB and LEACD had moderate and similar DPPH radical scavenging activities. Also REACA, REACC and LEACD showed better ABTS radical scavenging activities when compared with garlic acid while RBCA LEACA, LEACR and LEACD had similar ABTS radical scavenging activities when compared with garlic acid.

Nitric oxide radical scavenging assay showed that LEACA and LEACD had excellent nitric oxide radical scavenging activity which was better than that of garlic acid. REACB, REACC had similar scavenging activity to that of garlic acid. RCBA, REACE, REACF and LEACB had moderate and comparable nitric oxide radical scavenging activities.

The result of the hydroxyl radical scavenging assay revealed that REACA, REACB, REACC, REACE, and LEACE had hydroxyl radical scavenging that was better than garlic acid. LEACB had similar activity to that of garlic acid. The result of metal chelating assay revealed that RBCA, REACA, REACB, REACC, REACE, LEACA

and LEACD had remarkable metal chelating activities that was even better than that of garlic acid, a standard anti-oxidant.

The result of the anti-hypertensive assay showed that LEACA, LEACB, LEACC, LEACD had better anti hypertensive assay when compared with captopril a standard anti-hypertensive drug. LEACD gave the best anti-hypertensive activity when compared with the root extract, REACA and REACC gave a better anti-hypertensive activity. The compound from the root extract, REACA and REACC gave a better anti-hypertensive activity when compared with captopril. REACB gave a moderate anti-hypertensive activity when compared to captopril a standard ACE inhibitor.

The role played by reactive oxygen species (ROS) in pathological and physiological processes is paramont. (Afanas'ev, 2011 and Bisbal *et al*., 2010). Mitochondria is the primary source of ROS production and the primary target for their damaging effects (Lisa *et al*., 2009). Reactive oxygen species include supoxide anion, hydrogen peroxide (H_2O_2), Hydroxyl radicals (OH^-). The activities of these radicals are checked by anti-oxidant defence mechanism such as catalase, supoxide dismutase, glutathione peroxidase and peroxidoxins in the body cells (Marchi *et al*, 2013).

Build-up of ROS in vascular wall and reduction in the Nitric Oxide (NO) bio availability play a major role in endothelial dysfunction and atherogenesis (Lee *et al.*, 2012) they cause damage to endothelial cell and vascular smooth muscle cells. ROS and reactive nitrogen species (RNS) alter the sub-endothelial lipid and leads to atherosclerosis (Stocker and Keaney, 2004).NADPH oxidase plays important role in redox signaling events involved in hypertension, atherosclerosis, endothelial activation, angiogenesis and endothelial dysfunctions (Violi *et al*, 2009).

The functional association between NADPH oxidase and renin angiotensin system can explain the linkage between oxidative stress and hypertension (Puddu *et al*, 2008). Study by Lane *et al.* (2008) showed that consumption of anti-oxidants especially vitamin E & C protected against cardiac-ischemia-reperfusion injury and cardiovascular diseases (CVDs) in animal models. Antioxidants control of oxidative stress was ascertained as a veritable targets for CVDs prevention and therapy (Marchi *et al*, 2013).

A study by Rotenberg *et al*., (1990) suggested that anti-oxidant polyphenols in fruits, vegetables and plant derived beverages such as coffee and tea can increase anti-

oxidant capacity of the body and ameliorates diseases induced by oxidative stress through prevention of mitochondria alteration while totally and partially protecting the cells against the deleterious effects of oxidative stress.

Inclusion of anti-oxidant in the diet, and consumption of plants rich in anti-oxidants, vegetable, fruits, red wine, olive oil can increase anti-oxidant capacity and can ameliorate oxidative stress- induced cellular damage in cardiovascular diseases (Riccioni *et al*., 2012, Sari *et al*., 2010). Findings from this study proved that the isolated compounds are rich in phenols and are proven anti-oxidants and free radical scavengers and are excellent candidates for hypertension management.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The *in vitro* antioxidant, anti-inflammatory and anti-hypertensive activities of *P. americana,* leaf, bark and root extracts were also demonstrated by the ability of the three plant parts extracts to reduce pro- inflammatory molecules NFkB. These was more pronounced in the root and leaf extracts than the bark.

In vitro anti oxidant, anti- inflammatory and anti-hypertensive activities of *P. americana* leaf, bark and root extracts had variable DPPH radical scavenging, hydroxyl radical scavenging, nitric oxide scavenging, metal challenging ad iron reducing power activities. This study also demonstrated anti-inflammatory of the crude methanol extract and fractions and they were found to have strong anti-inflammatory activity comparable to indomethacin, a standard anti-inflammatory drug and Quercetin, a standard flavonoid. The extract and fraction exerted their anti-inflammatory activities through Lipoxigenase inhibition pathway. This makes them a better anti- inflammatory drugs because of the selective inhibition of lipoxigenase (LOX) enzyme and absence of undesirable gastric irritation and bleeding that is associated with non-steroidal anti-inflammatory drugs that mostly inhibit cyclo-oxygenese-1-enzyme. The invitro anti-hypertensive study on the extracts also demonstrated anti-hypertensive activities of the plant's leaves, bark and root extracts by angiotensin converting enzyme inhibition comparable to that of captopril standard anti-hypertensive drug.

The leaves, stem bark and root of *P. americana*were found to be rich in phenolic compounds which make them good antioxidant and free radical scavenger. Interestingly, they have high safety margin with median lethal dose (LD_{50}) of 3,162.27, 5477.23 and 3,463.91 mg/kg respectively for the leaves, bark and root extracts.Qualitative phytochemical analysis of the root extract showed the presence of

the following secondary metabolites: Alkaloids, glycosides, flavonoids, phenols, saponins, triterpenes and phytosterols.

This study also demonstrated *in vivo* anti-hypertension, anti-oxidant and anti-inflammatory effect with different mechanisms of action. The leaves, stem bark and root crude extracts had anti-hypertensive effects, however, the root gave the best result at 200mg/kg followed by the leaves extract and then the bark. Their effects are comparable to that of lisinopil, a standard angiotensin converting enzyme inhibitor and anti-hypertensive drug. They also protected the cardiac and renal tissues from hypertension-induced organ damage and arrhythmia possibly by free radical scavenging activities on cardiac and renal hydrogen peroxide, supoxide anion, myalondealdehyde (MDA) and protein carbonyl. It also potentiate the antioxidant defence mechanism as demonstrated by significant improvement in reduced glutathione, non-protein thiol, protein thiol, supoxide dismutare, glutathione trasferase and glutathione peroxidase in the cardiac and renal tissues. The*in vivo*anti-inflammatory actions of *P. americana,* leaf, bark and root were also demonstrated by the ability of the extracts of the three plant parts to reduce pro-inflammatory molecules such as NFkB. These were more pronounced in the root and leaves extracts than the bark.

In this study, I also isolated 11 pure compounds from butanol fraction of the root and ethyl acetate fractions of the leaf and root. These compounds include RBCA, LEACA, LEACB, LEACE, LEACD, REACA, REACB, REACC, REACD, REACE, and REACF. These were demonstrated to have profound antioxidant, anti-inflammatory and anti-hypertensive effects. The antioxidant effects were by free radical scavenging anti-inflammatory by lipoxygenase inhibition and anti-hypertensive activity by angiotensin converting enzyme inhibition.

I therefore conclude that the plant secondary metabolites and isolated compounds are good candidates for management of hypertension and amelioration hypertension-induced cardiac and renal damage.

6.2 Recommendation

I recommend that the structural elucidation of the isolated compounds be done and the identified compounds synthesized for clinical trials and drug development for hypertensive and inflammatory disease conditions management. I also recommend that the effect of extracts treatment on electrolytes profile should be investigated in future research.

6.3 Contribution to Knowledge

This work contributed to knowlegde through the following:

1) Determination of the *in* vitro antioxidant, anti-inflammatory and anti-hypertensive activities of the leaf, stem bark and root extracts of *Persea americana.*
2) Quantitatative and qualitative analyses of the *Persea americana* leaf, stem bark and root extracts and the discovery of secondary metabolites and phenolic content of the extracts.
3) Determination of the LD50 of 3162.27, 3463.91 and 5477.23 mg/kg, respectively for the leaf, bark and root extracts of *Persea americana*
4) Isolation of eleven bioactive phytocomponds from the leaf and root extracts which were coded RBCA, REACA, REACB, REACC, REACD, REACE, REACF, LEACA, LEACB, LEACC, LEACD
5) Determinaion of the *in vitro* antioxidant, anti-inflammatory and antihypertensive activities of the bioactivephytocompounds of the leaf, and root extracts of *Persea americana.*
6) This study found theanti-hypertensive effect of the crude methanol of the bark by Angiotensin Converting Enzyme inhibition was the best with more potency than captopril, a standard antihypertensive. This was followed by the crude methanol root extract while thatof the leaf was the least in activity.
7) From this study, it was found that all the crude extracts and fractions had anti-inflammatory activity comparable to that of indomethacin a standard non teroidal anti-inflammatory drug while the N-Butanol leaf, ethyl acetate bark and root fractions had better potency than indomethacin and comparable activities with Quercetin.

8) The anti inflammatory activities of the extracts, fraction and pure metabolite as reported in this study were by COX-2 inhibition, this showed that they are not associated with gastric irritation and are more tolerated in the gastrointestinal tract. They are, therefore very safe to administer by oral administration.
9) This study also found that the crude extact of the leaf, bark and root had wide safety margin from the $LD_{50}$and can safely be used without toxicity to human and animal.
10) The isolated pure compounds had variable anti oxidant activities compared to gallic acid with LEACA, LEACB and REACCgiving better antioxidant activities than gallic acid. REACC had better antioxidant activity than gallic acid in all the assays.
11) The extracts were reported to correct cardiac arrhythmia in hyprtension and they are therefore cardio-protective

REFERENCES

Abreu, D., Sousa, P., and Pinto, F.J. (2018). "Cardiovascular Disease and High Blood Pressure Trend Analyses from 2002 to 2016 : After the Implementation of a Salt Reduction Strategy." *BMC Public Health* 18(722) : 1–9.

Abu-Soud, H.M. and Hazen, S.L. (2000). Nitric oxide is a physiological substrate for mammalian peroxidases. J. Biol Chem 275 (48): 37524e32.

Adeboye, J.O., Fajoyonyomi, M.O., Makinde, J.M., Taiwo, O.B. (1999). A preliminary study on hypotensive activity of *P. amencana* leaf extract in anaesthetized, normotensive rats. Fitoterapia Vol 70: 15-20.

Adefegha, S. A., and Oboh, G. (2016). "Antioxidant and Inhibitory Properties of Clerodendrum Volubile Leaf Extracts on Key Enzymes Relevant to Non-Insulin Dependent Diabetes Mellitus and Hypertension." *Integrative Medicine Research* 10 (4): 521–33. http://dx.doi.org/10.1016/j.jtusci.2015.10.008.

Afanas'ev, I. (2011). ROS and RNS signalling in heart disorders, could antioxidants treatment be successful? Oxidation Medicine and cellular longevity vol 1:1-13.

Akinmoladun, A.C., Ibuku, E.O., Dan-Oloe, I.A. (2007). Phytochemical constituents and anti-oxidant properties of extracts from leaves of *Chromoluena odorata, Sci Res Essays.*2007; 2(6): 537-44.

Alimi A.A and Ashafa A.O.T. (2017). An invitro evaluation of the anti-oxidant and anti-diabetic potential of Sutherladia montane E. Phillips and RA Dyer Leaf extracts. Asian Pacific Journal of Tropical Biomedicine. 7 (9): 765-772

Anti, B.S., Okolon, J.E., Okon, P.A. (2005). Hypoglycaemic activities of aqueous leaf extracts of *Persea amencana* mill. Ind. J. Pharmacol. 37: 325-326.

Balsch, A.C.M., Urban, H., Ruiz, A.N. (2004). Endothelium dependent vasorelaxing activity of aqueous extracts of lyophilised seeds of *Casimiora edulis*. (AECE) on rat mesenteric arterial bed. J Ethnopharmacol. 95:163-167.

Baradaran, A., Nasri, H. and Rafieian-Kopaei, M. (2014). Oxidative Stress and Hypertension: Possibility of Hypertension Therapy with Antioxidants. J. Res Med Sci. 19(4): 358-367.

Beevers, G., Lip, G.Y. and O'Brien, E. (2001). ABC of hypertension: The pathophysiology of hypertension. See comment in PubMed Commons below BMJ 322: 912-916.

Benjamin, E.J., Larson, M.G., Keyes, M.J., Mitchell, G.F., Vasan, R.S.(2004). Clinical correlates and heritability of flow-mediated dilation in the community: the Framingham Heart Study. See comment in PubMed Commons below Circulation 109: 613-619.

Beutler E.O., Duron B., Kelly M. (1963) Improved method for the determination of glutathione. J lab Clin. Med. 61: 882-888

Bezáková, L., Grancăi, D., Obložinsky, M., Vanko, M., Holková, I, Pauliková, I., Garaj, V. and Gáplovskŷ, M. (2007): Effects of flavonoids and cynarine from Cynara cardunculus L., on lipoxygenase activity. Acta Facul Pharm Univ Com. Tomus Liv. 48-52

Birben, E., Sahiner, U.M., Sackesen, C., Erzurum, S. and Kalayci, O. (2012). Oxidative Stress and Antioxidant Defense. WAO Journal; 5:9–19.

Bisbal, C., Lambery, K. and Avigner, A. (2010). Antioxidants and glucose metabolism disorders, Current opinion in clinical Nutrition and Metabolic care. Vol 13. (4) 439-446

Bost, J.B. Smith, N.J.H.and Crane, J.H. (2013). History, distribution and uses. In The Avocado: Botany, Production and Uses; Schaffer, B.A., Wolstenholme, B.N., Whiley, A.W., Eds.; CABI: Wallingford, UK, 2013; pp. 10–30.

Botting, R. (2003). COX-1 and COX-3 inhibitors. Thromb Res. 110(5–6):269–72.

Brai, B.I., Odetola, A.A and Agomo, P.U. (2007). Hypoglycemia and Hypocholesterolemic Potential of *Persea americana* leaf extracts. Journal of Medicinal Food, 10 (2): 356-360.

Bovic, P.J. (2001). The role of phytosterols and phytosterolins in immune modulation: a review of the past 10 years. *Curr Opin Clin Nutr Metab Care* 4: 471-5.

Brand-Williams, W., Cuveleir, M.E. and Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. LWT-Food SciTechnol. 28(1):25–30.

Brook R.D. and Julius, S. (2000). Autonomic imbalance, hypertension and cardiovascular risk. Am J. Hypertens.; 13: 1125-1225. [PMID: 11073878].

Bryan, J. (2009). From snake venom to ACE inhibitor — the discovery and rise of captopril. The Pharmaceutical Journal. Vol. 282, p 45517.

Buettner G.R. (2011). Superoxide dismutase in redox biology: the roles of superoxide and hydrogen peroxide. Anticancer Agents Med Chem; 11(4):341–6.

Carretero O.A. and Oparil S. (2000). Essential hypertension: part II: treatment. See comment in PubMed Commons below Circulation 101: 446-453.

Chobanian A.V., Bakris G.L., Black H.R., Cushman W.C., Green L.A., Izzo J.L., Jones D.W., Materson B.J., Oparil S., Wright J.T., Jr. Roccella, E.J. and the National High Blood Pressure Education Program Coordinating Committee. (2003). Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. Hypertension; 42: 1206 –1252.

Clairborne A. (1985) Catalase activity. In: Greenward R.A., editor. Handbook for Methods for Oxygen Radical Research. Boca Roton, FL: CRC press. Pp283-284

Coca-Robinot D., Fabregate R., Sanchez-Largo E., Fernandez E., Marquez J et al. (2005) Clinical, biochemical and cardiovascular implications of endothelial dysfunction in the dermal microcirculation. American journal of hypertension 18: 177A-178A.

Costanzo P., Perrone-Filardi P., Petretta M., Marciano C., Vassallo E. (2009). Calcium channel blockers and cardiovascular outcomes: a meta-analysis of 175,634 patients. J Hypertens 27:1136-1151.

Dai, X. et al. (2018). “Mechanisms in Hypertension and Target Organ Damage : Is the Role of the Thymus Key ? (Review).” *Int J Mol Med* 42(1): 3–12.

Delacroix, S., Chokka, R.C. and Worthley, S.G. (2014). Hypertension: Pathophysiology and Treatment. J Neurol Neurophysiol 5: 250. doi:10.4172/2155-9562.1000250.De Wet, B. (2011) Medicinal plants and human health. S Afr Pharm J. 78(6):38–40.

Dias, M.I.; Sousa, M.J.; Alves, R.C.; Ferreira, I.C.F.R. (2016). Exploring plant tissue culture to improve the production of phenolic compounds: A review. Ind. Crops Prod. 82: 9–22.

Drieshbach, A.W. (2015). Pathphysiology of Essential Hypertension. Pathophysiology of Hypertension.

Drury R.A., Wallington, E.A., Canscerson R. (1976) Calton’s Histopathological Techniques, 4th Edition, Oxford, London, New York: Oxford University Press.

Duni, A., Vassilios L., Karolos-pavlos R., and Evangelia D. (2018). “Chronic Kidney Disease and Disproportionally Increased Cardiovascular Damage : Does Oxidative Stress Explain the Burden ?” *Oxid Med Cell Longev*: 1–18.

Dzau V.J., Bernstein K. and Calermajer D. (2001). Working group on tissue angiotensin: the relevance of tissue angiotensin converting enzyme: manifestations in mechanistic and end point data. Am J Cardiol 88: 1L-20L.

Edward, F. G. and Dennis, G. W. (1994). “*Persea Americana* Avocado”. Fact Sheet ST-435: A Series from Environmental Horticulture Department, Florida Cooperative Extension Service.

Ellman G.L (1959). Tissue Sulphhydryl groups. Arch Biochem. Biophys. 82:70-77

Elmer P.J., Obarzanek E., Vollmer W.M., Simons-Morton D., Stevens V.J, et al. (2006) Effects of comprehensive lifestyle modification on diet, weight, physical fitness, and blood pressure control: 18-month results of a randomized trial. Annals of internal medicine 144: 485-95.

Etuk E.U. (2006). A review of medicinal plants with hypotensive or antihypertensive effects. Journal of Medical Sciences, 6: 894-900.

Fozard J.R., and Part M.L. (1991). Haemodynamic responses to NG – Monomethyl-L-arginine in spontaneously hypertensive and normotensive wistar-kyoto rats.Br J. pharmacol – 102 (4): 823-826.

Frishman W.H., Beravol P. and Carosella C. (2009). Alternative and complementary medicine for preventing and treating cardiovascular disease. Dis. Mon. 55; 121-192.

Gareth, B. Gregory, Y.H.L. and Eoin O. (2001). The Pathphysiology of Hypertension. ABC of Hypertension. Biotechnology Medical Journal. Vol 322 (7291): 912-916.

Garrat D.C. (1964). Quantitative Analysis of drugs Japan 3rd Edn; Chapman and Hall, Japan pp 456-458

Giles T.D., Berk B.C., Black H.R., Cohn J.N., Kostis J.B. and Izzo Jr J.L. (2005). Expanding the definition and classification of hypertension. J Clin Hypertens (Greenwich);7(9): 505–12.

Go A.S. (2013). Heart Disease and Stroke Statistics-2013 Update: A Report From the American Heart Association. Circulation 127: E841-E841.

Habib W.H., Pabst M.J., Jacoby W.B. (1974)Glutathione-S-transferase activities. The enzymatic effect in mecapturic acid formation. J.Biol. Chem. 249:130-139

Haramaki, N., Packer, L., Assadnazari, H. and Zimmer, G. (1993). “Cardiac recovery during post-ischemic reperfusion is improved by combination of vitamin E with dihydrolipoic acid,” Biochemical and Biophysical Research Communications, vol. 196. (3): 1101–1107.

Hermann M., Flammer A. and Luscher T.F. (2006). Nitric oxide in hypertension. J. Clin. Hypertens. (Greenwich) 8: 17-29.

Hollenberg N.K. (2003). The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). Major outcomes in high-risk hypertensive patients randomized to angiotensin-converting enzyme inhibitor or calcium channel blocker vs diuretic. Curr Hypertens Rep 5: 183-185.

Huang, WY, Cai YZ., Zhang Y. (2010). Natural Phenotic compounds from medicinal herbs and dietary plants: potential use for cancer prevention. *Nutr Cancer* 62: 1-20.

Huo, Wenqian et al. (2010). "Kidney Injury Molecule-1 (KIM-1): A Novel Kidney-Specific Injury Molecule Playing Potential Double-Edged Functions in Kidney Injury." *Transplantation Reviews* 24(3): 143–46. http://dx.doi.org/10.1016/j.trre.2010.02.002.

Jaffrey S.R and Snyder S.H. (1995) "Nitric oxide: a neural messenger,"Annual Review of Cell and Developmental Biology, vol.11: 417–440.

Jin L. et al. 2017. "Gallic Acid Reduces Blood Pressure and Attenuates Oxidative Stress and Cardiac Hypertrophy in Spontaneously Hypertensive Rats." *Scientific Reports* 7 (1): 1–14. http://dx.doi.org/10.1038/s41598-017-15925-1.

Johnson, R. J., Miguel A. L., Sánchez-lozada, L.G. and Rodriguez-iturbe, B. (2018). The Discovery of Hypertension : Evolving Views on the Role of the Kidneys , and Current Hot Topics." *Am J Physiol Renal Physiol* 308(3): 1–23.

Kabel, A. M. (2014). "Free Radicals and Antioxidants: Role of Enzymes and Nutrition." *World Journal of Nutrition and Health* 2(3): 35–38.

Karthikeyan, S., Gobinanand, K., Pradeep, K., Mohan, C. & Balasubramanian, M. (2006). Biochemical changes in serum lung, heart and spleen tissues of mice exposed to inhalation of mosquito repellent material vapour. Journal of environmental biology. 27:355-358.

Kannan, N., Kunnathur M. S., and Chandrasekaran G.(2013). "Protective Effect of Acacia Nilotica (L.) against Acetaminophen-Induced Hepatocellular Damage in Wistar Rats." *Advances in Pharmacological Sciences* 2013.

Klein, H.H., Pich, S., Lindert-Heimberg, S., Nebendahl, K and Niedmann, P. (1993) "Failure of chronic, high-dose, oral vitamin E treatment to protect the ischemic, reperfused porcine heart," Journal of Molecular and Cellular Cardiology, vol. 25 (1): pp. 103–112.

Kolackowska E and Paul Kubes (2013). Neutrophil recruitment and function in health and inflammation. Nature reviews immunology 13: 159-175.

Kusano, C. and Bucalen F. (2011). "The biology of nuclear factor kappa beta (NFkB) in health and pathology." *Journal of Arts, Science & Commerce* 2(3): 53–59.

Lahogue V., Rehel K., Taupin L., Haras D. and Allaume P (2010). A HPLC-UV method for the determination of angiotensin-1-converting enzyme (ACE) inhibitory activity. Food Chem 118: 870-875.

Laursen, J.B., Rajagopalau, S., Galis, Z., Tarpey, M., Freeman, B.A. and Harrison, D.G. (1997). Role of superoxide in angiotensin II induced but not catecholamine-induced hypertension. Circulation; 95:588-93. [PMID:9024144]

Law M.R., Morris J.K and Wald N.J. (2009). Use of blood pressure lowering drugs in the prevention of cardiovascular disease: meta-analysis of 147 randomised trials in the context of expectations from prospective epidemiological studies. BMJ; 338:b1665.

Lee, S., Lee, I., and Mar, W. (2003). Inhibition of inducible nitric oxide synthase and cycloxygenase-2 activity by 1, 2, 3, 4, 6-penta-O-galloyl-beta-D-glucose in murine macrophage cells. Arch Pharm Res. 26:832–9.

Lee L, M., Margaritis, K. M., Channon, A., and Antoniades C. (2012). "Evaluating oxidative stress in human cardiovascular disease: methodological aspects and considerations," Current Medicinal Chemistry, Vol. 19 (16). 2504-2520.

Levenson J.W., Skerrett P.J and Gaziano J.M. (2002). Reducing the global burden of cardiovascular disease: the role of risk factors. Prev Cardiol. 5(4):188-99.

Lin L., Lv S., and Li B. (2012). Angiotensin-I-converting enzyme (ACE)-inhibitory and antihypertensive properties of squid skin gelatin hydrolysates. *Food Chem* 131: 225-230.

Lisa, F. Kaludercic, N., Carpi, A., Menabo, R., and Gior-gio, M. (2009). "Mitochondria and vascular pathology," Pharmacological Reports, vol. 61 (1): 123–130,

Loga-Zec S., Asceric M., Loga-Andrijic N., Kapetanovic B. and Zerem E. (2014). The incidence of Anti-Hypertensive drug-induced side effects in patients with Diabetes Mellitus Type 2 and Hypertension. Med Arch; 68(6): 372-375.

Loria V., Dato, I., Graziani, F., Biasucci, L.M. (2008). Myeloperoxidase: a new biomarker of inflammation in Ischemic heart disease and acute coronary syndromes, Mediators of Inflammation, volume. Article ID 135625, 4 pages.

Lowry, O.H., Rosebrough N.I., Farr A.L., Randal R.J. (1951) Protein measurement with folin phenol reagent.Biol. Chem.; 193: 265-275

Luscher T.F; Vanhoutte P.M. (1986). Endothelium dependent contractions to acetylcholine on the aorta of spontaneously hypertensive rat. Hypertension. 8: 344-348.

MacNee, W. (2001) Oxidative stress and lung inflammation in airway diseases. Eur. J. Pharm. 429: 195–207.

Maisuthisukul P., Venketeshwer R. (2012) Phenolic constituents and anti-oxidant properties of some Thai plants, phytochemicals global perspective of their role in nutrition and health.USA: Intech; p.548.

Marchi E.D., Baldassari F., Bononi A., Wlechcowski M.R., Pinton P. (2013). Oxidative stress in cardiovascular diseases and obesity: Kole of P66 shc and protein kinase C. oxidative medicine and cellular longevity 2013 (1): 564961.

Masuyama, T., Yamamoto, K., Sakata, Y., Doi, R., Nishikawa, N., Kondo, H., Ono, K., Kuzuya, T., Sugawara, M., Hori, M.(2000). Evolving changes in Doppler mitral flow velocity pattern in rats with hypertensive hypertrophy. J. Am. Coll. Cardiol. 36: 2333– 2338.

McCarthy, F.P., Kingdom, J.C., Kenny, L.C., Walsh S.K. (2011). Animal models of preeclampsia: Uses and limitations. Placenta 32(6):413–19.

Mark, A.L. (1996) The sympathetic nervous system in hypertension: a potential long-term regulator of arterial pressure. J HypertensSuppl 14: S159-165.

Makipour, S., Kanapuru, B. and Ershler W.B. (2008).Unexplained Anemia in the Elderly. Semin Hematol.; 45 (4): 250–254.

Martindale J.L., and Holbrook, N.J., (2002). Cellular response to oxidative stress: signaling for suicide and survivaL. Journal of Cellular Physiology, Vol 192 (1): 1- 15.

Masella, R., Di Benedetto, R., Vari R., Filesi C. and Giovanni C. (2005). Novel mechanisms of natural antioxidant compounds in biological systems. Involvement of glutathione and glutathione related enzymes. J. Nutr. Biochem. 16: 577-586.

Mayet, J. and Hughes, A. (2003). Cardiac and vascular pathophysiology in hypertension. See comment in PubMed Commons below Heart 89: 1104-1109.

Messerli, F. H., Stefano, F. R., and Sripal B. (2017). "The Transition From Hypertension to Heart Failure." *Heart Failure* 5(8): 543–51.

Misra H.P., and Fridovich I. (1972) The role of Supoxide anion in the auto oxidation of epinephrine and a simple assay for supoxide dismutase. J.Biol. Chem. 247: 3170-3175

Morton, J.F. (1987). Fruits of Warm Climates. Creative resource system, Inc., Winterville, NC and Center for New Crops & Plant Products, Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, IN 8:91-102,

Mruk, D.D., Silvestrini, B., Mo, M.Y and Cheng, C.Y. (2002). Antioxidant superoxide dismutase–a review: its function, regulation in the testis, and role in male fertility. Contraception; 65(4):305–11.

Nandhini, .S. (2014). Essential Hypertension –A Review Article. J. Pharm. Sci. &Res. Vol. 6 (9): 305-307.

National Heart Foundation of Australia. Guideline for the diagnosis and management of hypertension in adults. (2016). *Guideline for the Diagnosis and Management of Hypertension in Adults*.

Nguyen, T., Paul N., and Cecil, B. P. (2009). "The Nrf2-Antioxidant Response Element Signaling Pathway and Its Activation by Oxidative Stress " *J. Biol.*

Chem. 284(20): 13291–95.

Nilvetjdis, R., van Nood, E., van Hoorn, D.E.C., Boelens, E.G., van Norren, K., van Leeuwen, P.A.M. (2001). Flavonoids: a review of probable mechanisms of action and potential application. Am J Clin Nutr. 74:418–25.

Nurdin R., Nikmah, U. D., and Bohari, (2018). Phytochemical and Antioxidant Activity of Avocado Leaf Extract (*Persea americana*Mill.). *Asian Journal of Scientific Research, 11: 357-363.*

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 (a-amylase and a-glycosidase) and hypertension (Angiotensin 1 Converting) in vitro. *J. Funct Foods* 4: 450-458.

Oboh, G., Puntel, R.L., Rocha, J.B.T.I., (2007) Hot pepper *(Caspsicum annum, Tepin and Caspiscum chinese, Habanero*) prevents Fe induced lipid peroxidation in brain- in vitro, Food chemistry, Vol 102, (1), 178-185

Ogah, O.S., Okpechi, I., Chukwuonye, I.I., Akinyemi, J.O., Onwubere, B.J., Falase, A.O., Stewart, S. and Sliwa, K (2012). Blood pressure, prevalence of hypertension and hypertension related complications in Nigerian Africans: A review. World journal of cardiology; 4:327-340

Ogden, L.G., He J., Lydick E. and Whelton P.K. (2000). Long-term absolute benefit of lowering blood pressure in hypertensive patients according to the JNC VI risk stratification. Hypertension; 35: 539–543.

Ojewole, J.A.O., Kamadyaapa D., Gondiwe, M., Moodley K., Mujabayane. C. (2007) cardiovascular effect of *Persea americana*. Mill (Lauraceae) avocado aqueous laef extract in experimental animal. Cardiovascular journal of Africa Vol 18 (2): 69-76.

Ojewole, J.A.O., and Amabeoku, G. (2006) Anticonvulsant effect of *Persea americana* Mill (Lauraceae) (Avocado) leaf aqueous extract in mice. Phytotherapy Research. Vol. 20 (8): 696 - 700

Okwu, D.E. (2004). Phytochemical and vitamin content of indigenous spices of South Eastern Nigeria, *J Sustain Agric Environ*: 6: 30-4.

Olaleye S.B., Adaramoye O.A., Erigbali P.P. Adeniyi O.S. (2007) Lead exposure increases oxidative stress in the gastric mucosa of HCl/ ethanol-exposed rats. World J. Gastroenterol.13: 5121-5126

Olukunle J.O., Jacobs E.B., Ajayi O.L., Biobaku K.T. Abatan M.O. (2014) Toxicological evaluation of the aqueous extract of Acalypha wilkesisia in Wistar albino rats. J. Complement Integr. Med.1-4.

Omobowale, T.O. and Nottidge H.O. (2011). Clinical and Molecular studies on *Babesia canis* infection in dogs in Nigeria. (Ph.D Thesis, University of Ibadan).

Onuigbo, M., Nneoma A., Mohan S., and Karen R.. (2017). "Acute Kidney Injury in Heart Failure Revisited—The Ameliorating Impact of 'Decongestive Diuresis' on Renal Dysfunction in Type 1 Acute Cardiorenal Syndrome: Accelerated Rising Pro B Naturetic Peptide Is a Predictor of Good Renal Prognosis." *Journal of Clinical Medicine* 6(9): 82. http://www.mdpi.com/2077-0383/6/9/82.

Otte J., Shalaby S.M., Zakora M., Pripp A.H. and El-Shabrawy S.A. (2007). Angiotensin converting enzyme inhibitory activity of milk protein hydrolysates: Effect of substrate, enzyme and time of hydrolysis. International Dairy Journal, 17: 488:-503.

Owolabi, M.A., Jaja, S.I., and Coker, H.A. (2010). Bioactivity of the Phytoconstituents of the Leaves of *Persea americana*. Journal of medical plants research volume 4 (12): 1130-1135.

Pacher, P., Beckman, J.S. and Liaudet, L. (2007). Nitric oxide and peroxynitrite in health and disease. Physiol Rev; 87(1):315–424. doi: 10.1152/physrev.00029.2006.

Palmer R.M.J., Ferrige A.G., Mocada S.(1973). Nitric oxide release account for the endothelium-derived relaxing factor. Nature Vol. 27: 524 -536.

Palmer, R.M.L., Rees D.D., Aston DS, Moncada S.(1988). L- arginine is the physiological precursor for the formation of nitric oxide in endothelium-dependent relaxation. Biochem. Biophys Res. Commun 15 (3):1251-1256.

Panza J.A., Garcia C.E., Kilcoyne C.M., Quyyumi A.A and Cannon R.O. (1995). 3rd. Impaired endothelium-dependent vasodilation in patients with essential hypertension. Evidence that nitric oxide abnormality is not localized to a single signal transduction pathway. Circulation 91: 1732-1738.

Phaniendra A., Jestadi D.B, and Periyasamy,. L (2015) "Free radicals: properties, sources, targets, and their implication in various diseases," Indian Journal of Clinical Biochemistry, Vol. 30, no. 1: 11–26.

Piepho R.W and Beal J. (2000) An overview of antihypertensive therapy in the 20th century. See comment in PubMed Commons below J ClinPharmacol 40: 967-977.

Poniedzialek- csakowska, E., Maranlak, B., Kimber – Trojinar, Z. Bozena Leszczynska- G., Oleszczuk, J. (2011). Nitric oxide in normal and pre-eclamptic pregnancy, Current Pharmaceutical Biotechnology Vol 12 (5): 743-749.

Praschat. Bimlesh K., Mandeep K., Gurpreet K., Harleen K., (2011). Phytochemical screening and extaction: a review. Int Pharm Sci. 1 (1): 98-106.

Puddu, P G. M. Puddu, E. Cravero, M. Rosati, and A. Muscari, (2008). "The molecular sources of reactive oxygen species in hypertension," Blood Pressure, vol. 17(2): 70–77.

Rafiq S., Anand S. and Roberts R.(2010). Genome-wide association studies of hypertension: have they been fruitful? J Cardiovasc Transl Res. 3 (3): 189-96

Rajappa, Rashmi, Venugopalreddy Bovilla, and Subbarao V. Madhunapantula. (2017). "Naturally Occurring Nrf2 Activators in the Management of Diabetes." *Nutri Food Sci Int J*2(4).

Ridker PM, Thuren T, Zalewski A, Libby P. (2011). Interleukin-1beta inhibition and the prevention of recurrent cardiovascular events: rationale and design of the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS). Am Heart J. 162: 597–605.

Rees. D.D., Palmer R.M.J., Monacada S. (1989).Role of endothelial derived nitric oxide in the regulation of blood pressure. Proc Nat. Acad. Sci. 86. 3375-3378.

Re, R., Pellegrinni, 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.

Riccioni, G., Speranza, L., Pesce, M., Cusenza, S. D'Orazio, N and Glade, M.J. (2012). "Novel phytonutrient contributors to antioxidant protection against cardiovascular disease," Nutrition, vol. 28(6): 605–610.

Rodrigues M. R. C., Rondina, D., Araujo, A.A., Souza, A. l.. Nunespinheiro, D.C., Fernades, A. A.O., Ibipina, F.L. (2011). Reproductive and metabolic responses of ewes fed dehydrated cashew apple bagasse during the postpartum period. Arq. Bras. Med. Vet. Zootec, Vol 63 (1): 171-179.

Rimoy G.H., Justin-Temu M.I and Nilay C. (2008). Prescribing patterns and Cost of Antihypertensive drugs in Private Hospitals in Dar es Salaam, Tanzania. East Cent Afr J Pharm Sci. 11:69-73.

Rotenberg, S.A., Krauss, R. S., Borner,C. M. B., and Weinstein, I. B. (1990). Characterization of a specific form of protein kinase C overproduced by a C3H 10T(1/2) cell line," Biochemical Journal, vol. 266(1): 173–178.

Rotruck J.T., Pope J.L.,Ganther H.E., et al. (1973) Selenum: biochemical role as a component of glutathione peroxidase. Sci. 179; 588-590

Sabri A., Hughie H.H and Lucchesi P.A. (2003). Regulation of hypertrophic and apoptotic signaling pathways by reactive oxygen species in cardiac myocytes. Antioxid Redox Signal; 5: 731–740.

Sadik, C.H.D., Sies, H, Schewe T. (2003). Inhibition of 15-lipoxygenase by flavonoids: structure activity relation and mode of action. Biochem Pharm. 65:773–81.

Samb B, Desai N, Nishtar S, Mendis S, Bekedam H, Wright A, *et al*. (2010). Prevention and management of chronic disease: a litmus test for health systems strengthening in low-income and middle-income countries. Lancet; 376:1785–1797.

Sander M., Chavoshan B. and Victor R.G. (1999). A large blood pressure-raising effect of nitric oxide synthase inhibition in humans. See comment in PubMed Commons below Hypertension 33: 937-942.

Sandor B. and Thomas T. (2012). Micro RNAs in Hypertension: Mechanisms and Therapeutic Targets. Journal of Springer Science; Curr hypertens Rep, 14:79-87

Sanoski C.A (2009). Aliskiren: an oral direct renin inhibitor for the treatment of hypertension. Pharmacotherapy 29: 193-212.

. Sari, I., Baltaci, Y. and Bagci, C. (2010). "Effect of pistachio diet on lipid parameters, endothelial function, inflammation, and oxidative status: a prospective study," Nutrition, vol. 26, no. 4: 399– 404.

Saxena P.R. (1992) Interaction between the renin-angiotensin-aldosterone and sympathetic nervous system. J. Cardivas Pharm.19 (6) S80-88

Smith, R. E. (2016). "The Role of the Nrf2 / ARE Antioxidant System in Preventing Cardiovascular Diseases." *diseases* 2: 1–20.

Stocker, R. and J. F. Keaney Jr. (2004). "Role of oxidative modifications in atherosclerosis," Physiological Reviews, vol. 84, no. 4: 1381– 1478, 2004.

Suzuki Y.J., Forman H.J. and Sevanian A. (1997). Oxidants as stimulators of signal transduction. Free Radic Biol Med 22: 269-285.

Tadhani M.B., Pitel V.H., Subhash K. (2007). Invitro-antioxidant activities of *Stevia rebaudiana* leaves and callus. Jounal of Food Composition and Analysis, 20:323-329.

Temitope, O.A, Akinola, M.O., Aladejana, O.M. and Ogunlade, A.O. (2017). Efficacy of Essential oils from Persea Americana Stem, Bark and seed extracts J. Appl. Microbiol Biochem. Vol. 1. (3): 1-12.

Thurman, J.M., and Shrier, R.W. (2003). Comparative effects of angiotensin converting enzyme inhibitors and angiotensin receptor blockers on blood pressure and the kidney. The American Journal of Medicine. 114 (7): 588-598.

Todorich, B., Olopade, J.O., Surguladze, N. (2011): The mechanism of vanadium-mediated developmental hypomyelination is related to destruction of oligodendrocyte progenitors through a relationship with ferritin and iron. Neurotox Res.19: 361–373.

Takimoto E. and Kass D.A. (2007). Signalling pathways of Reactive Oxygen Species and cardiovascular hypertrophy and remodelling. Hypertension. 49:241-248

Tom B., Garrelds I.M., Scalbert E., Stegmann A.P., Boomsma F., Saxena P.R. and Danser A.H. (2003). ACE-Versus chymase dependent angiotensin II generation in human coronary arteries: a matter of efficiency? Arterioscle Thromb Vasc Biol. 23(2): 251-6.

Townsend D.M and Tew K.D. (2003). The role of glutathione-S-transferase in anti-cancer drug resistance. Oncogene 22, 7369–7375.

Turgolu, A. and Gezer, K. (2007). Antioxidant & antimicrobial activities of Lactiporus- sulphureus (Bull) Murrill, Food chemistry, Vol 101, (1): 267-273

Tuteja N., Singh M.B., Misra M.K. (2001). Molecular mechanisms of DNA damage and repair: progress in plants. Crit Rev Biochem Mol Biol, 36: 337–97

USDA plant profile. (2012). United States Department of Agriculture. National Resources Conservation Service. April 2012.

Varshney R. and Kale R.K. (1990) Effect of camodulin antagonist on radiationinduced lipid peroxidation in microsomes. Int. J. Biol. 158: 733-741

Vavrova L., Kocik M. and Zak A. (.2014). Human catalase: its polymorphisms, regulation and changes of its activity in different diseases. Folia Biol (Praha); 60(4):153–67.

Violi, F., Basili, S., Nigro, C., and Pignatelli, P. (2009). "Role of NADPH oxidase in atherosclerosis," Future Cardiology, vol. 5(1): 83–92.

Watt J.M., Breyer - Brandwijk M.G. (1962). The medicinal and poisonous. Plants of southern and Eastern Africa 2nd edition. Edinburgh, E and S Livingstone 53 – 54.

Weber M.A., Schiffrin E.L., White W.B., Mann S., Lindholm L.H. (2014). Clinical practice guidelines for the management of hypertension in the community: a statement by the American Society of Hypertension and the International Society of Hypertension. J Clin Hypertens (Greenwich) 16: 14-26.

Wink, D.A., Kasprzak, K.S., Maragos, C.M., Elespuro, R.K., Misra, M., Dunam T. (1991). DNA deaminating ability and genotoxicity of nitric oxide and its progenitors, science, Vol 254: 5034

Winterbourn C.C. (2013). The biological chemistry of hydrogen peroxide. Methods Enzymol; 528:3–25. doi: 10.1016/B978-0-12-405881-1.00001-X.

Wolfe .K. Wu X., Liu R.H. (2003). Antioxidant activity of appe peels. J. Agric Food Chem. 51:609-614.

Wolff S.P. (1994) Ferrous ion oxidation in the presence of Ferric ion indicator xylenol orange for the measurement of hydroperoxides. Method Enzymol. 233:182-189.

World Health Organization (2002) Reducing risks, promoting healthy life. The world health report. 5: 113-118

World Health Organization (2013) A global brief on hypertension. Geneva, Switzerland: World Health Organization.http://www.who.int/cardiovascular_diseases/publications/global_brief_hypertension/en/index.html. [Accessed 14 January 2015]

Wood, T.M., Bhat, K.M.I. (1988). Methods for measuring cellulose activities, methods in enzymology, Vol 160: 87-112.

Wu G, Fang Y.Z, Yang S., Lupton J.R. and Turner N.D. (2004). Glutathione metabolism and its implications for health. J Nutr; 134(3):489–92.

Wu, Jing, and David G Harrison. (2015). "Oxidative Stress and Hypertension." In Blood Pressure and Arterial Wall Mechanics in Cardiovascular Diseases. 175–92.

Xia Y. and Zweier J.L. (1997) Measurement on myeloxiperoxidase in leucocyte containing tissues. Anal Biochem 245: 93-96.

Zalba G., Jose G.S., Moreno M. U., Fortuno M.A., Fortuno O.A. (2001) Oxidative stress in arterial hypertension: Role of NAD(P)H Oxidase. Hypertension 38: 1395- 1399.

APPENDIX

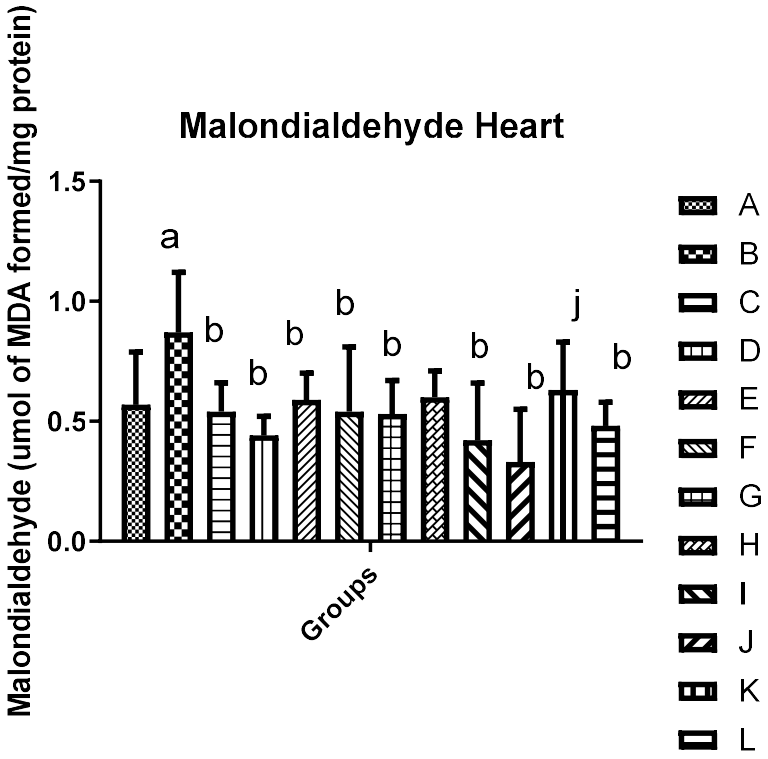


Figure 1: The MDA result of the heart of the hypertensive rats treated with *P. americana*extracts

L-NAME was given orally at 40mgkg^{-1}. Lisinopril was given at 0.28mgkg^{-1} and Extracts measured in mgkg^{-1}at the dosage of 100 (1), 200 (2) and 400 (3), respectively for the*Persea americana* leaf (PALE), bark (PABE) and root (PARE) extracts as given to groups B-K with L-NAME while A received distilled water and Lreceived L. NAME + Lisinopril.

Significance was measured at values presented as mean ± SEM. Groups. Significance was measured at $\alpha_{0.05}$, Means with superscript a - l showed significance when when compared with their respective groups A-L.

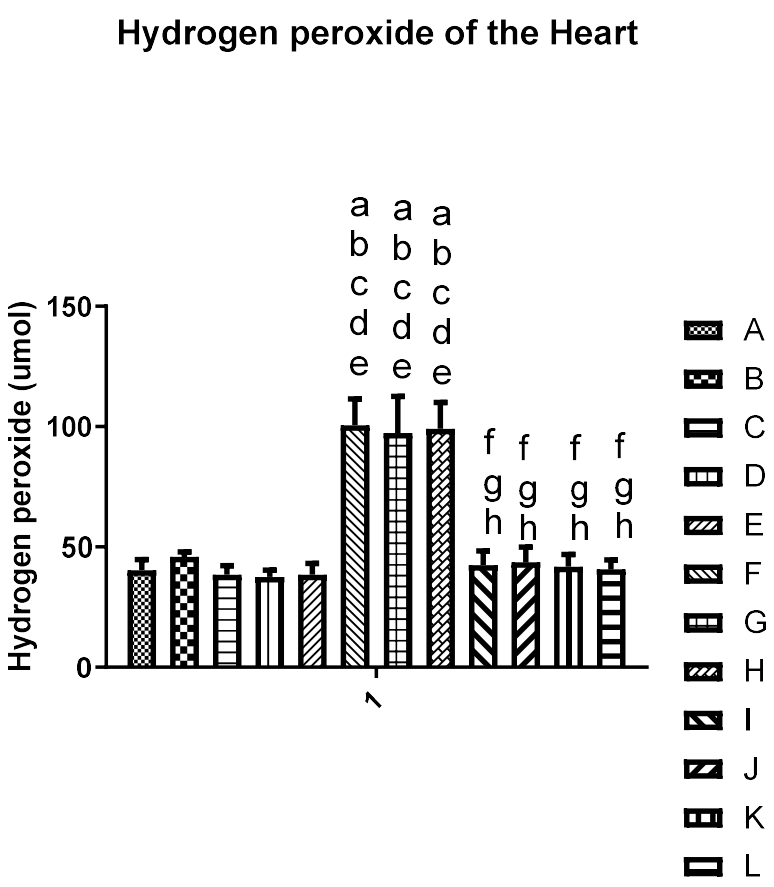


Figure 2: The Hydrogen peroxide result of the heart of the hypertensive rats treated with *P. americana*extracts

L-NAME was given orally at 40mgkg^{-1}. Lisinopril was given at 0.28mgkg^{-1} and Extracts measured in mgkg^{-1}at the dosage of 100 (1), 200 (2) and 400 (3), respectively for the*Persea americana* leaf (PALE), bark (PABE) and root (PARE) extracts as given to groups B-K with L-NAME while A received distilled water and Lreceived L. NAME + Lisinopril.

Significance was measured at values presented as mean ± SEM. Groups. Significance was measured at $\alpha_{0.05}$, Means with superscript a -l showed significance when compared with their respective groups A-L.

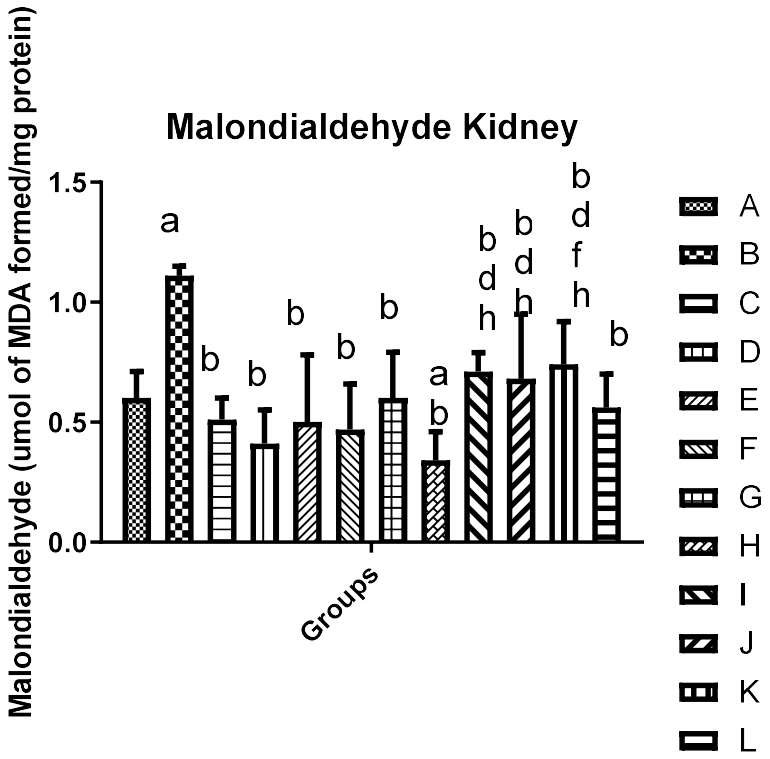


Figure 3: The MDA result of the kidney of the hypertensive rats treated with *P. americana*extracts.

L-NAME was given orally at 40mgkg^{-1}. Lisinopril was given at 0.28mgkg^{-1} and Extracts measured in mgkg^{-1}at the dosage of 100 (1), 200 (2) and 400 (3), respectively for the*Persea americana* leaf (PALE), bark (PABE) and root (PARE) extracts as given to groups B-K with L-NAME while A received distilled water and Lreceived L. NAME + Lisinopril.

Significance was measured at values presented as mean ± SEM. Groups. Significance was measured at $\alpha_{0.05}$, Means with superscript a - l showed significance when compared with their respective groups A-L.

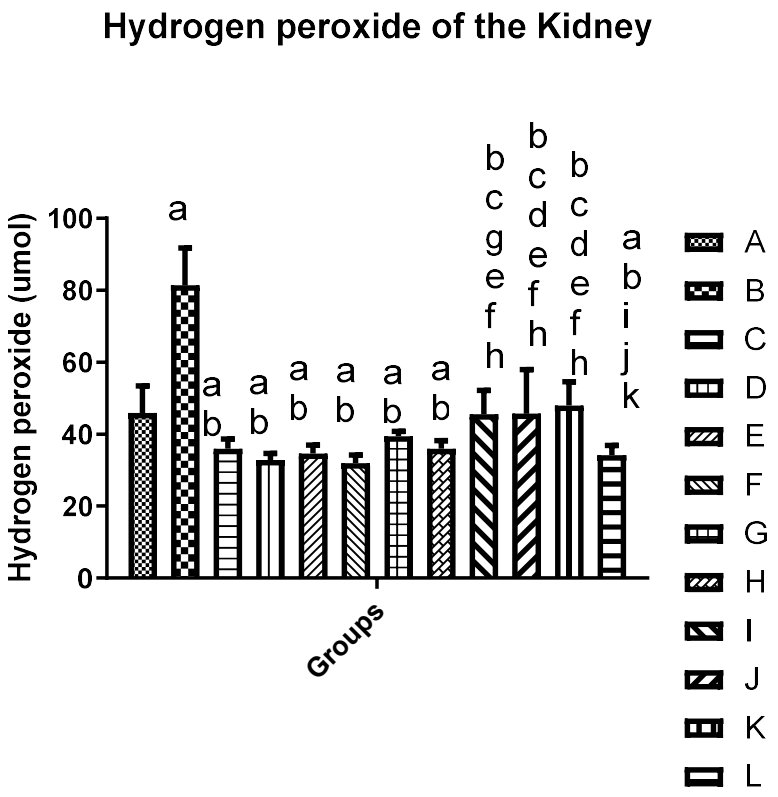


Figure 4: The Hydrogen peroxide result of the Kidney of the hypertensive rats treated with *P. americana*extracts

L-NAME was given orally at 40mgkg^{-1}. Lisinopril was given at 0.28mgkg^{-1} and Extracts measured in mgkg^{-1}at the dosage of 100 (1), 200 (2) and 400 (3), respectively for the*Persea americana* leaf (PALE), bark (PABE) and root (PARE) extracts as given to groups B-K with L-NAME while A received distilled water and Lreceived L. NAME + Lisinopril.

Significance was measured at values presented as mean ± SEM. Groups. Significance was measured at $\alpha_{0.05}$, Means with superscript a - l showed significance when compared with their respective groups A-L.

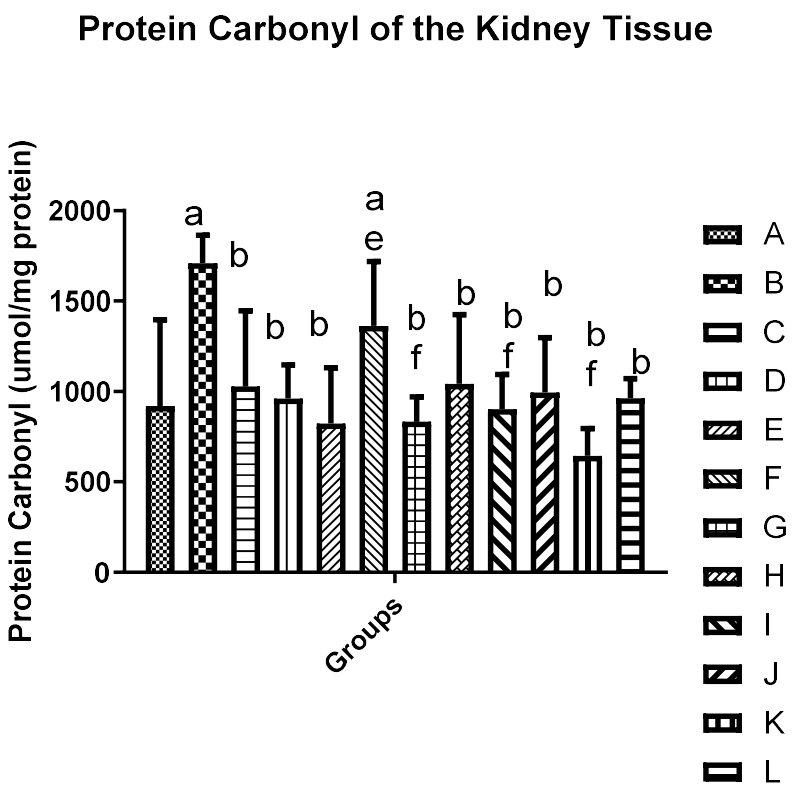


Figure 5: The Protein carbonyl result of the Kidney of the hypertensive rats treated with *P. americana*extracts

L-NAME was given orally at 40mgkg^{-1}. Lisinopril was given at 0.28mgkg^{-1} and Extracts measured in mgkg^{-1}at the dosage of 100 (1), 200 (2) and 400 (3), respectively for the*Persea americana* leaf (PALE), bark (PABE) and root (PARE) extracts as given to groups B-K with L-NAME while A received distilled water and Lreceived L. NAME + Lisinopril.

Significance was measured at values presented as mean ± SEM. Groups. Significance was measured at $\alpha_{0.05}$, Means with superscript a - l showed significance when compared with their respective groups A-L.

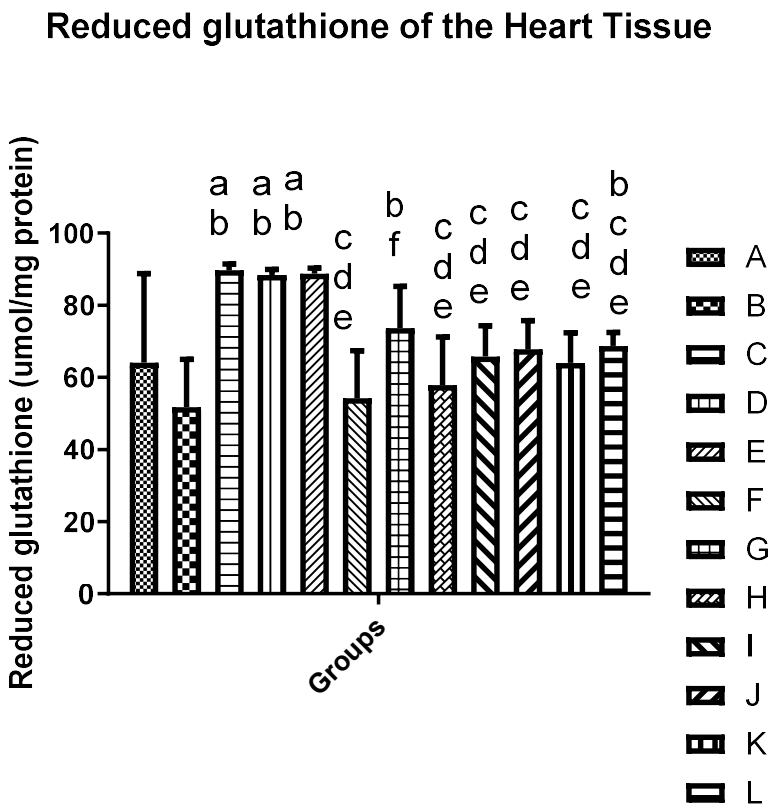


Figure 6: The GSH result of the Heart of the hypertensive rats treated with *P. americana*extracts.

L-NAME was given orally at 40mgkg^{-1}. Lisinopril was given at 0.28mgkg^{-1} and Extracts measured in mgkg^{-1}at the dosage of 100 (1), 200 (2) and 400 (3), respectively for the*Persea americana* leaf (PALE), bark (PABE) and root (PARE) extracts as given to groups B-K with L-NAME while A received distilled water and Lreceived L. NAME + Lisinopril.

Significance was measured at values presented as mean ± SEM. Groups. Significance was measured at $\alpha_{0.05}$, Means with superscript a - l showed significance when compared with their respective groups A-L.

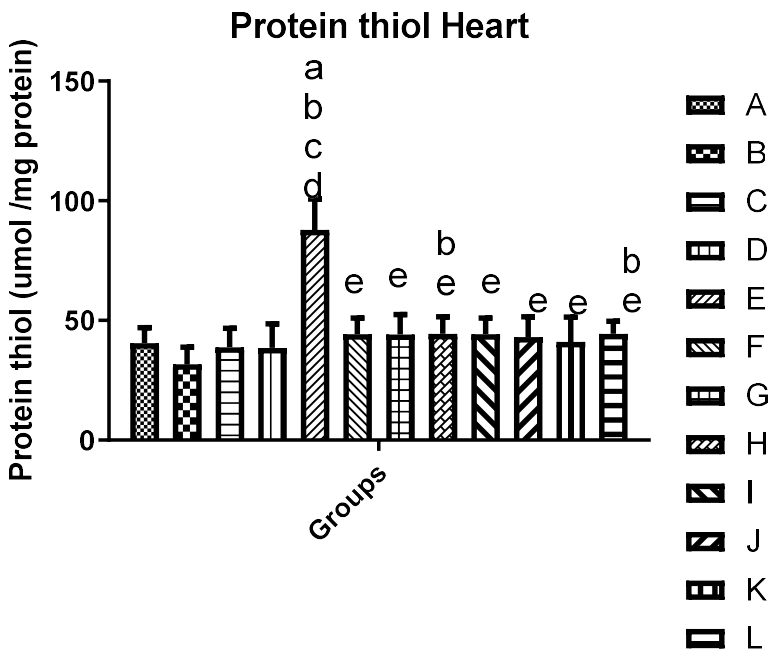


Figure 7: The Protein thiol result of the Heart of the hypertensive rats treated with *P. americana*extracts.

L-NAME was given orally at 40mgkg^{-1}. Lisinopril was given at 0.28mgkg^{-1} and Extracts measured in mgkg^{-1}at the dosage of 100 (1), 200 (2) and 400 (3), respectively for the*Persea americana* leaf (PALE), bark (PABE) and root (PARE) extracts as given to groups B-K with L-NAME while A received distilled water and Lreceived L. NAME + Lisinopril.

Significance was measured at values presented as mean ± SEM. Groups. Significance was measured at $\alpha_{0.05}$, Means with superscript a - l showed significance when compared with their respective groups A-L.

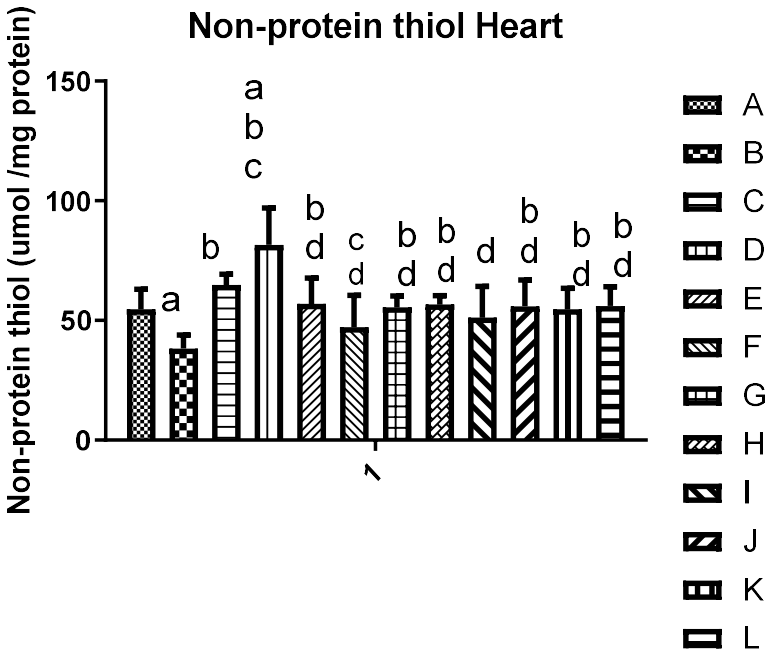


Figure 8: The Non-Protein thiol result of the Heart of the hypertensive rats treated with *P. americana*extracts

L-NAME was given orally at 40mgkg^{-1}. Lisinopril was given at 0.28mgkg^{-1} and Extracts measured in mgkg^{-1}at the dosage of 100 (1), 200 (2) and 400 (3), respectively for the*Persea americana* leaf (PALE), bark (PABE) and root (PARE) extracts as given to groups B-K with L-NAME while A received distilled water and Lreceived L. NAME + Lisinopril.

Significance was measured at values presented as mean ± SEM. Groups. Significance was measured at $\alpha_{0.05}$, Means with superscript a - l showed significance when compared with their respective groups A-L.

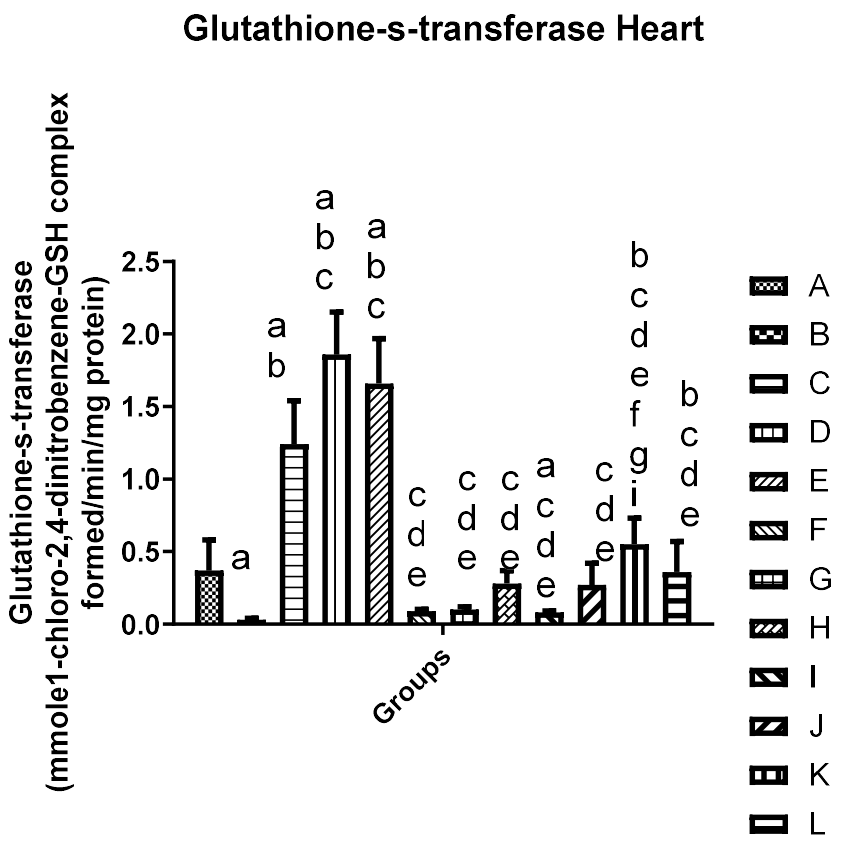


Figure 9: The GST result of the Heart of the hypertensive rats treated with *P. americana*extracts

L-NAME was given orally at 40mgkg^{-1}. Lisinopril was given at 0.28mgkg^{-1} and Extracts measured in mgkg^{-1}at the dosage of 100 (1), 200 (2) and 400 (3), respectively for the*Persea americana* leaf (PALE), bark (PABE) and root (PARE) extracts as given to groups B-K with L-NAME while A received distilled water and Lreceived L. NAME + Lisinopril.

Significance was measured at values presented as mean ± SEM. Groups. Significance was measured at$\alpha_{0.05}$, Means with superscript a – l showed significance when compared with their respective groups A-L.

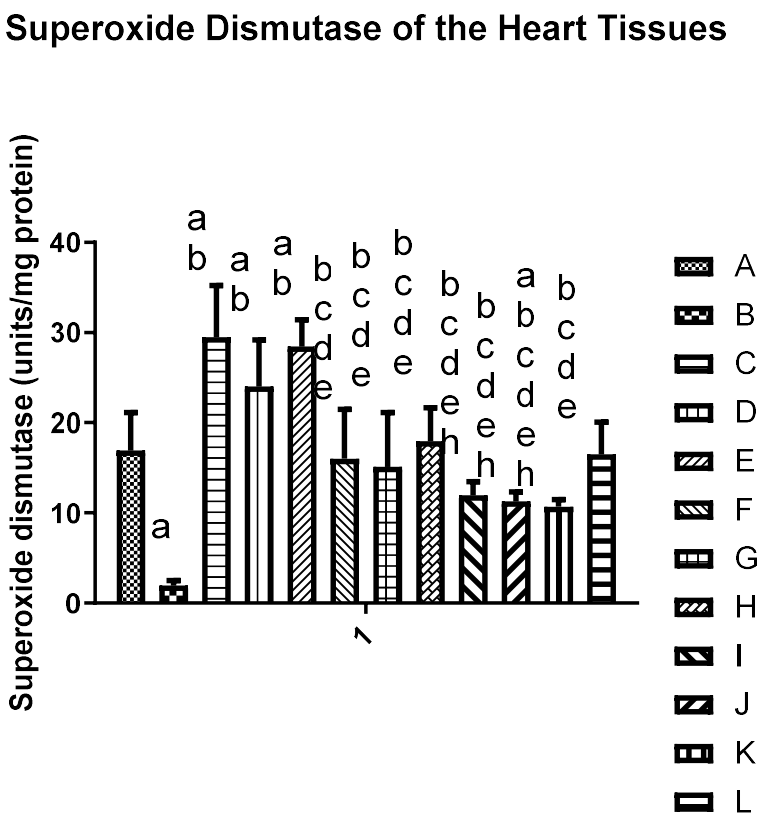


Figure 10: The SOD result of the Heart of the hypertensive rats treated with*P. americana*extracts.

L-NAME was given orally at 40mgkg^{-1}. Lisinopril was given at 0.28mgkg^{-1} and Extracts measured in mgkg^{-1}at the dosage of 100 (1), 200 (2) and 400 (3), respectively for the*Persea americana* leaf (PALE), bark (PABE) and root (PARE) extracts as given to groups B-K with L-NAME while A received distilled water and Lreceived L. NAME + Lisinopril.

Significance was measured at values presented as mean ± SEM. Groups. Significance was measured at $\alpha_{0.05}$, Means with superscript a - l showed significance when compared with their respective groups A-L.

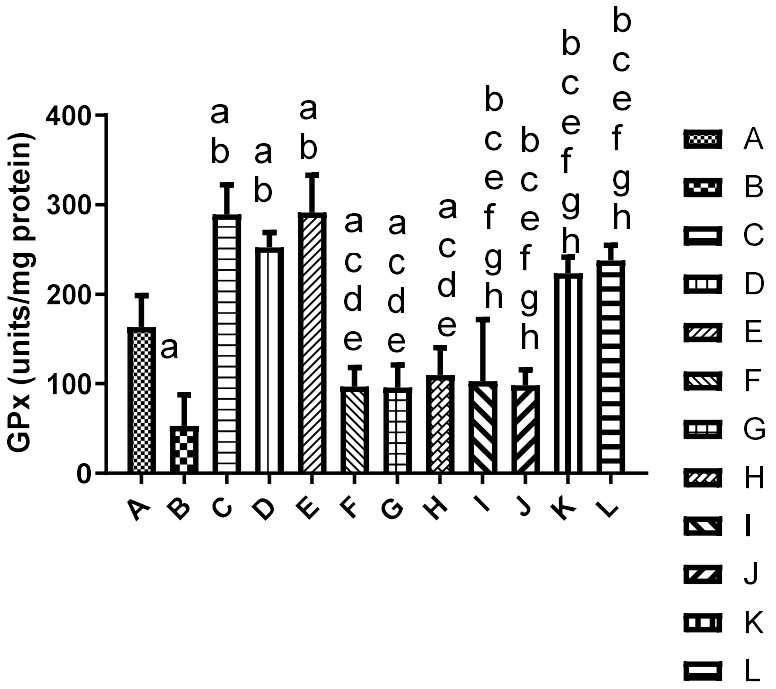


Figure 11: The GPx result of the Heart of the hypertensive rats treated with *P. americana*extracts

L-NAME was given orally at 40mgkg^{-1}. Lisinopril was given at 0.28mgkg^{-1} and Extracts measured in mgkg^{-1}at the dosage of 100 (1), 200 (2) and 400 (3), respectively for the*Persea americana* leaf (PALE), bark (PABE) and root (PARE) extracts as given to groups B-K with L-NAME while A received distilled water and Lreceived L. NAME + Lisinopril.

Significance was measured at values presented as mean ± SEM. Groups. Significance was measured at $\alpha_{0.05}$, Means with superscript a - l showed significance when compared with their respective groups A-L.

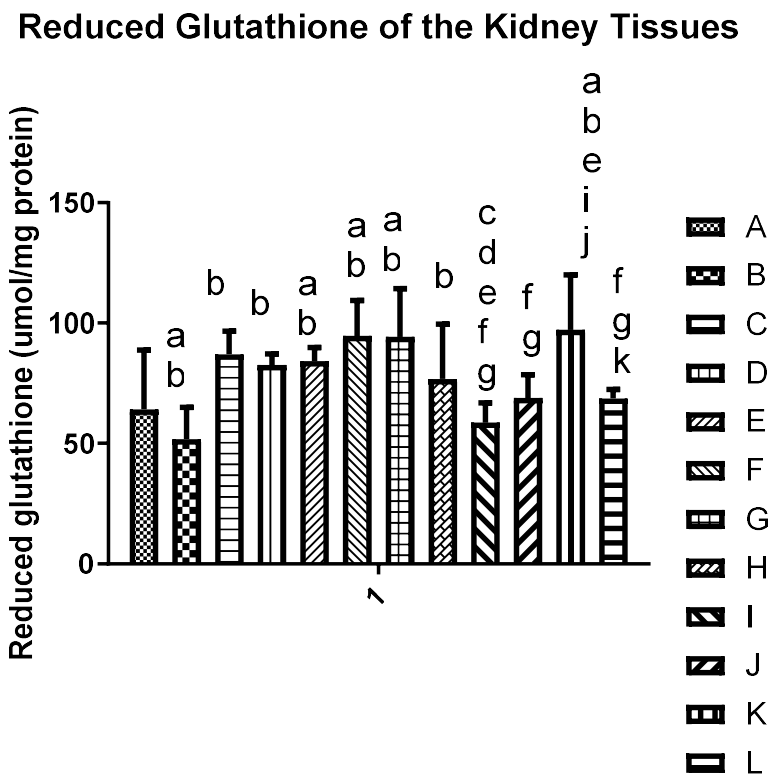


Figure 12: The GSH result of the Kidney of the hypertensive rats treated with *P. americana*extracts

L-NAME was given orally at 40mgkg^{-1}. Lisinopril was given at 0.28mgkg^{-1} and Extracts measured in mgkg^{-1}at the dosage of 100 (1), 200 (2) and 400 (3), respectively for the*Persea americana* leaf (PALE), bark (PABE) and root (PARE) extracts as given to groups B-K with L-NAME while A received distilled water and Lreceived L. NAME + Lisinopril.

Significance was measured at values presented as mean ± SEM. Groups. Significance was measured at $\alpha_{0.05}$, Means with superscript a - l showed significance when compared with their respective groups A-L.

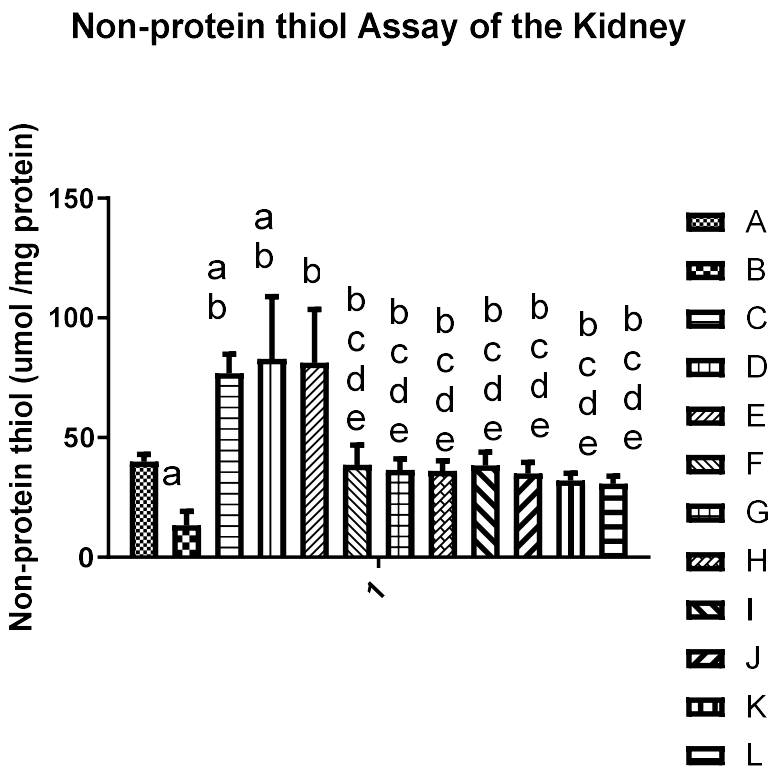


Figure 13: The NPT result of the kidney of the hypertensive rats treated with *P. americana*extracts.

L-NAME was given orally at 40mgkg^{-1}. Lisinopril was given at 0.28mgkg^{-1} and Extracts measured in mgkg^{-1}at the dosage of 100 (1), 200 (2) and 400 (3), respectively for the*Persea americana* leaf (PALE), bark (PABE) and root (PARE) extracts as given to groups B-K with L-NAME while A received distilled water and Lreceived L. NAME + Lisinopril.

Significance was measured at values presented as mean ± SEM. Groups. Significance was measured at $\alpha_{0.05}$, Means with superscripta - l showed significance when compared with their respective groups A-L.

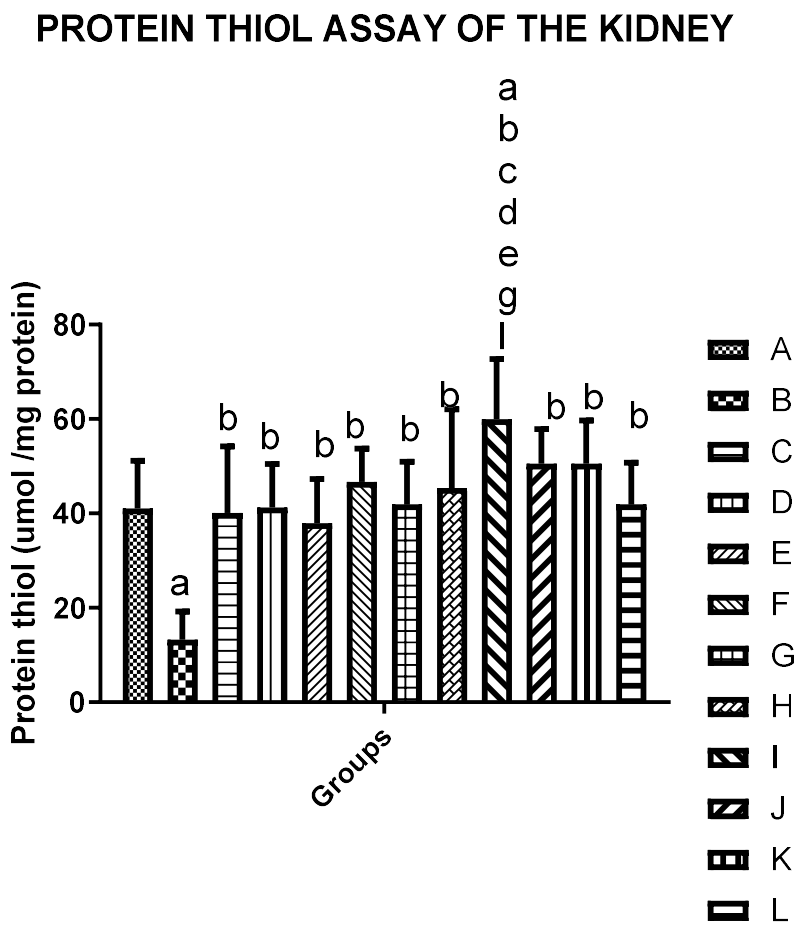


Figure 14: The Protein thiol result of the Kidney of the hypertensive rats treated with *P. americana*extracts

L-NAME was given orally at 40mgkg^{-1}. Lisinopril was given at 0.28mgkg^{-1} and Extracts measured in mgkg^{-1}at the dosage of 100 (1), 200 (2) and 400 (3), respectively for the*Persea americana* leaf (PALE), bark (PABE) and root (PARE) extracts as given to groups B-K with L-NAME while A received distilled water and Lreceived L. NAME + Lisinopril.

Significance was measured at values presented as mean ± SEM. Groups. Significance was measured at $\alpha_{0.05}$, Means with superscript a - l showed significance when compared with their respective groups A-L.

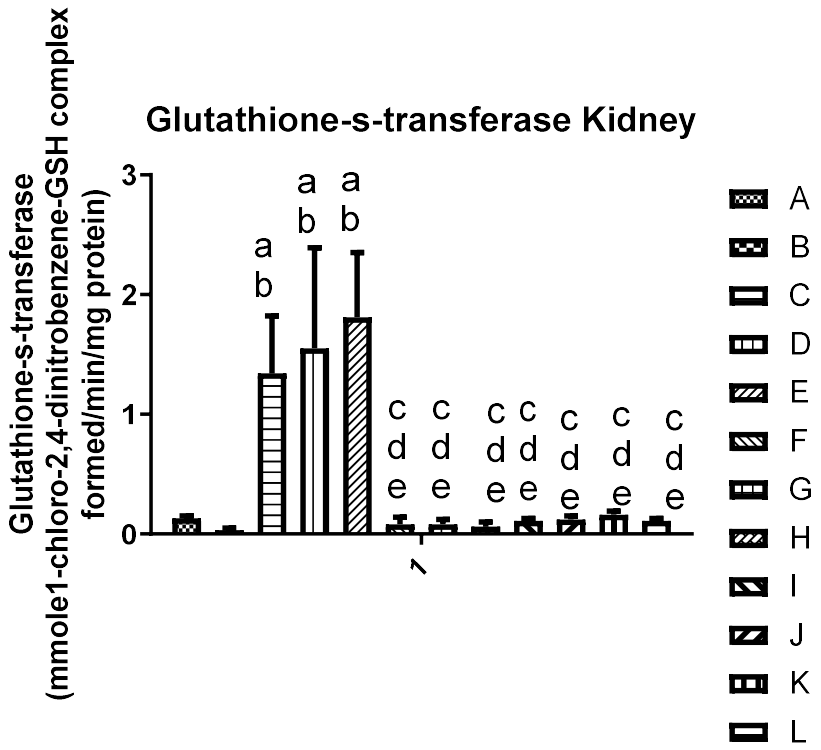


Figure 15: The GST result of the Kidney of the hypertensive rats treated with *P. americana*extracts

L-NAME was given orally at 40mgkg^{-1}. Lisinopril was given at 0.28mgkg^{-1} and Extracts measured in mgkg^{-1}at the dosage of 100 (1), 200 (2) and 400 (3), respectively for the*Persea americana* leaf (PALE), bark (PABE) and root (PARE) extracts as given to groups B-K with L-NAME while A received distilled water and Lreceived L. NAME + Lisinopril.

Significance was measured at values presented as mean ± SEM. Groups. Significance was measured at$\alpha_{0.05}$, Means with superscript a - l showed significance when compared with their respective groups A-L.

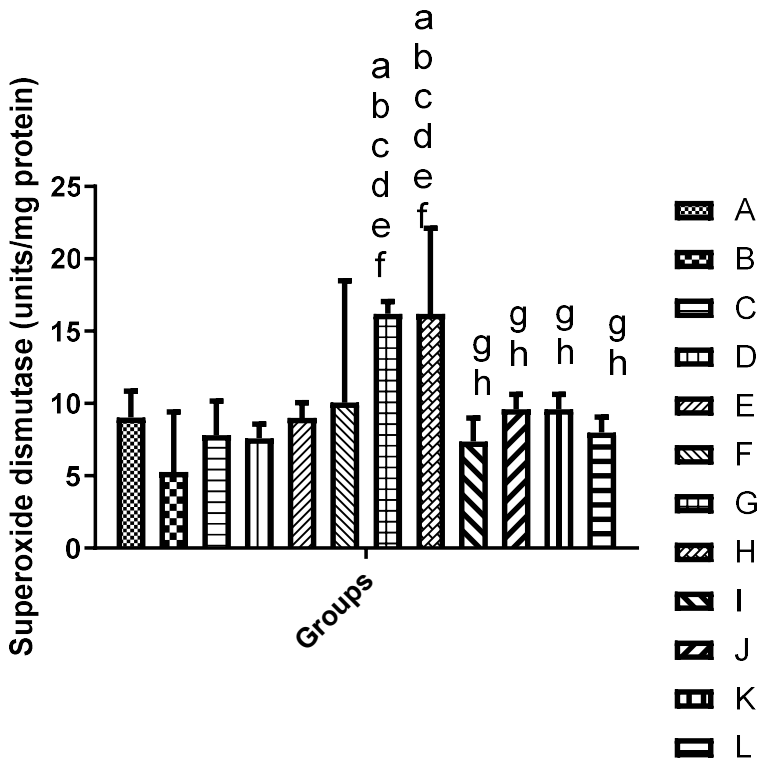


Figure 16: The SOD result of the Kidney of the hypertensive rats treated with *P. americana*extracts.

L-NAME was given orally at 40mgkg^{-1}. Lisinopril was given at 0.28mgkg^{-1} and Extracts measured in mgkg^{-1}at the dosage of 100 (1), 200 (2) and 400 (3), respectively for the*Persea americana* leaf (PALE), bark (PABE) and root (PARE) extracts as given to groups B-K with L-NAME while A received distilled water and Lreceived L. NAME + Lisinopril.

Significance was measured at values presented as mean ± SEM. Groups. Significance was measured at$\alpha_{0.05}$, Means with superscript a - l showed significance when compared with their respective groups A-L.

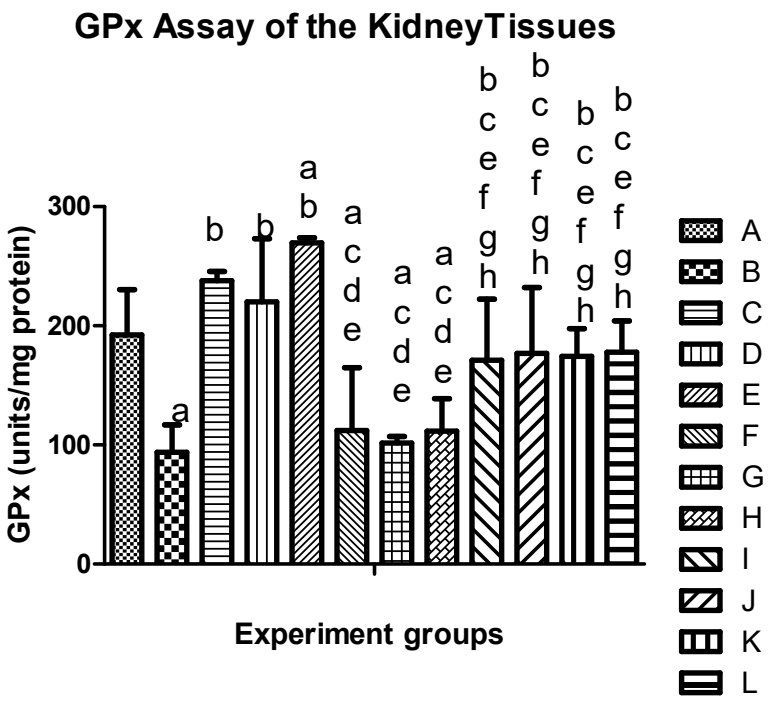


Figure 17: The SOD result of the Kidney of the hypertensive rats treated with *P.americana*extracts.

L-NAME was given orally at 40mgkg^{-1}. Lisinopril was given at 0.28mgkg^{-1} and Extracts measured in mgkg^{-1}at the dosage of 100 (1), 200 (2) and 400 (3), respectively for the*Persea americana* leaf (PALE), bark (PABE) and root (PARE) extracts as given to groups B-K with L-NAME while A received distilled water and Lreceived L. NAME + Lisinopril.

Significance was measured at values presented as mean ± SEM. Groups. Significance was measured at $\alpha_{0.05}$, Means with superscripta - l showed significance when compared with their respective groups A-L.

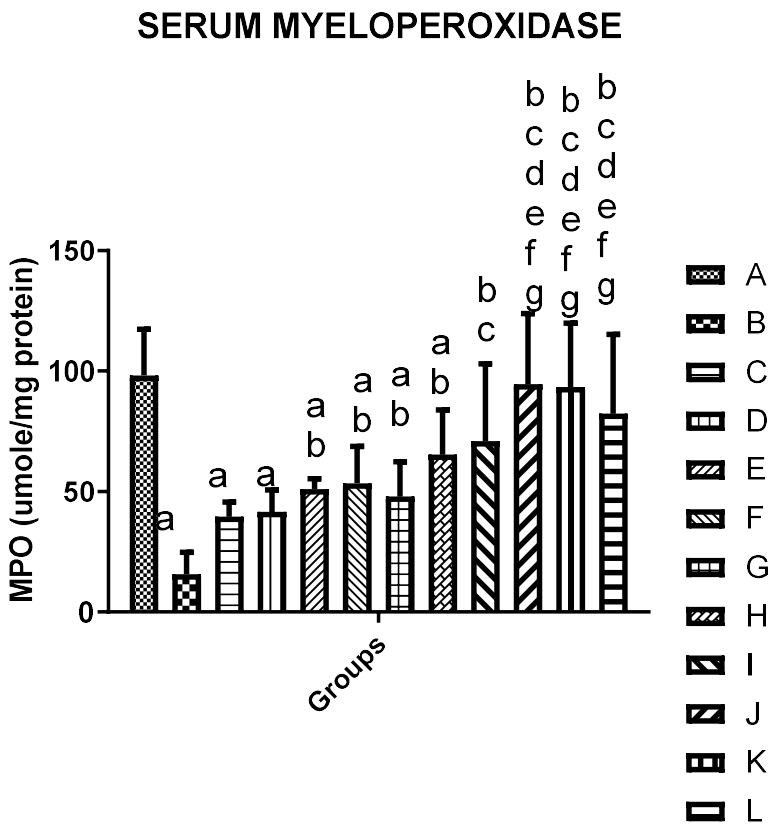


Figure 18: The MPO result of the Serum of the hypertensive rats treated with *P. americana*extracts.

L-NAME was given orally at 40mgkg^{-1}. Lisinopril was given at 0.28mgkg^{-1} and Extracts measured in mgkg^{-1}at the dosage of 100 (1), 200 (2) and 400 (3), respectively for the*Persea americana* leaf (PALE), bark (PABE) and root (PARE) extracts as given to groups B-K with L-NAME while A received distilled water and Lreceived L. NAME + Lisinopril.

Significance was measured at values presented as mean ± SEM. Groups. Significance was measured at $\alpha_{0.05}$, Means with superscript a - lshowed significance when compared with their respective groups A-L.

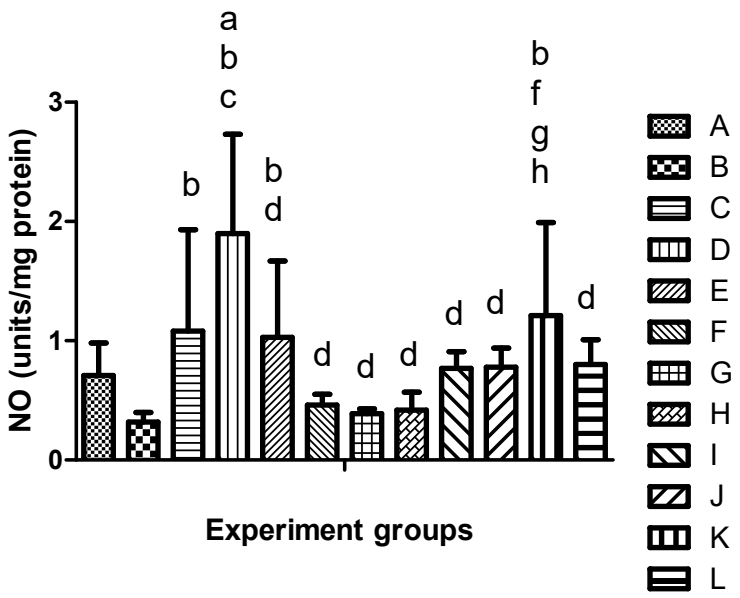


Figure 19: The Nitric oxide result of the Serum of the hypertensive rats treated with *P. americana*extracts.

L-NAME was given orally at 40mgkg^{-1}. Lisinopril was given at 0.28mgkg^{-1} and Extracts measured in mgkg^{-1}at the dosage of 100 (1), 200 (2) and 400 (3), respectively for the*Persea americana* leaf (PALE), bark (PABE) and root (PARE) extracts as given to groups B-K with L-NAME while A received distilled water and Lreceived L. NAME + Lisinopril.

Significance was measured at values presented as mean ± SEM. Groups. Significance was measured at $\alpha_{0.05}$, Means with superscript a – l showed significance when compared with their respective groups A-L.

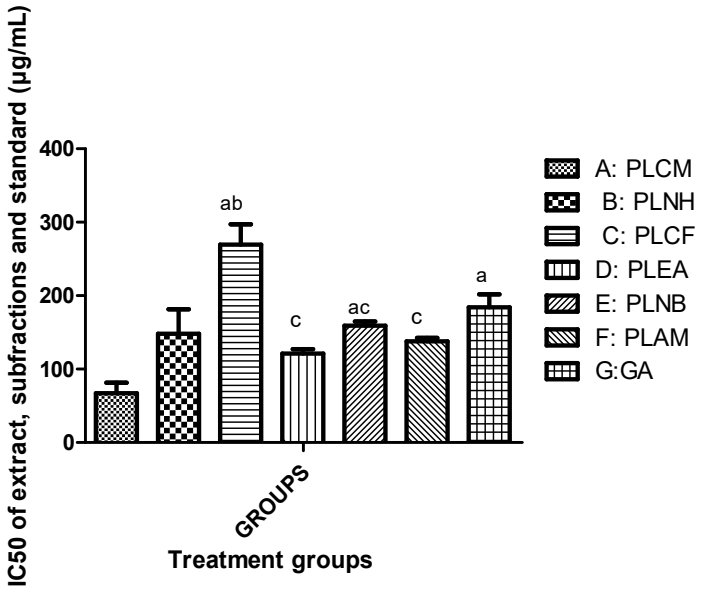


Figure 20: Showing the IC 50 values of *P. americana* leaf extract and fractions and gallic acid in metal chelating assay.

Group A: *P. americana* leaf crude methanol extract (PLCM) Group B: *P.americana* leaf N-Hexane fraction (PLNH), Group C: *P.americana* leaf Chloroform fraction (PLCF), Group D: *P.americana* leaf Ethylacetate fraction (PLEA), Group E :*P.americana* leaf N-Butanol fraction (PLNB), Group F : *P.americana* leaf Aqueous Methanol fraction (PLAM), Group G : Gallic acid (GA). Significance at $\alpha_{0.05}$

ABTS ASSAY RESULTS FOR P. AMERICANA LEAF EXTRACTS AND FRACTIONS

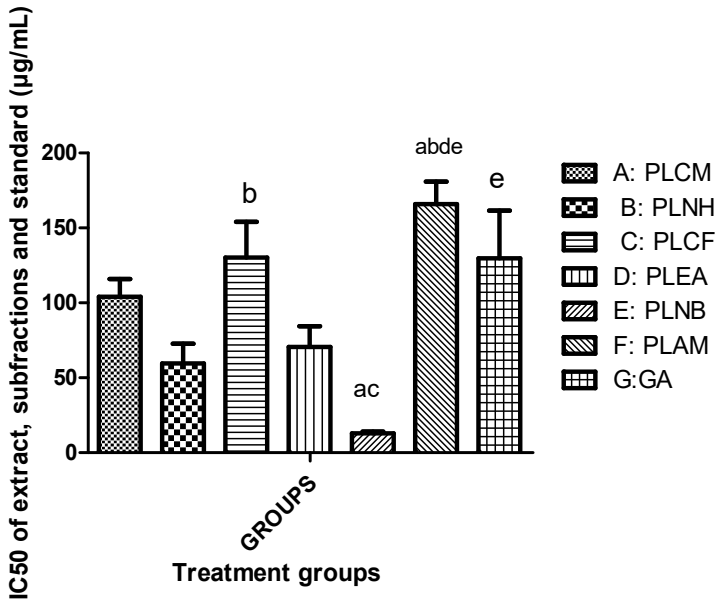


Figure 21: Showing the IC 50 values of *P. americana* leaf extract and fractions and gallic acid in ABTS assay

Group A: *P. americana* leaf crude methanol extract (PLCM) Group B: *P.americana* leaf N-Hexane fraction (PLNH), Group C :*P.americana* leaf Chloroform fraction (PLCF), Group D : *P.americana* leaf Ethylacetate fraction (PLEA), Group E : *P.americana* leaf N-Butanol fraction (PLNB), Group F : *P.americana* leaf Aqueous Methanol fraction (PLAM), Group G : Gallic acid (GA). Significance at $\alpha_{0.05}$

HYDROXYL RADICAL SCAVENGING ASSAY FOR P. AMERICANA LEAF EXTRACT AND FRACTIONS

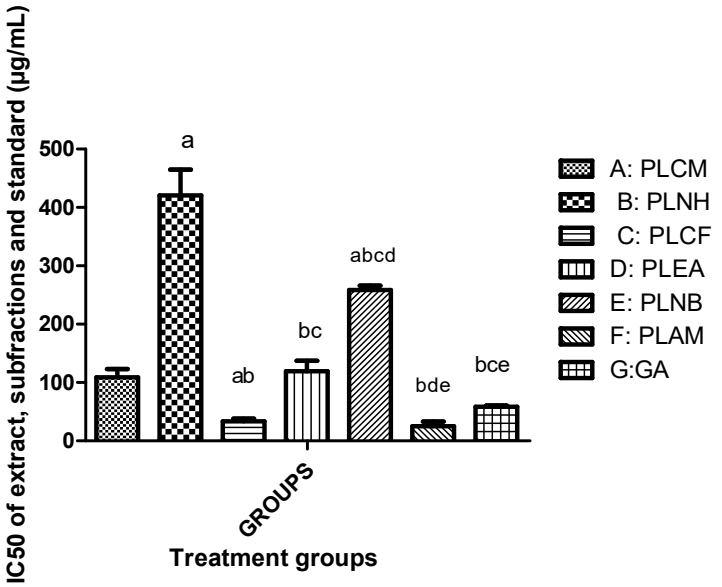


Figure 22: Showing the IC 50 values of *P. americana* leaf extract and fractions and gallic acid in hydroxyl radical scavenging assay.

Group A: *P. americana* leaf crude methanol extract (PLCM) Group B :*P.americana* leaf N-Hexane fraction (PLNH) , Group C : *P.americana* leaf Chloroform fraction (PLCF), Group D : *P.americana* leaf Ethylacetate fraction (PLEA), Group E : *P.americana* leaf N-Butanol fraction (PLNB), Group F : *P.americana* leaf Aqueous Methanol fraction (PLAM), Group G : Gallic acid (GA). Significance at $\alpha_{0.05}$

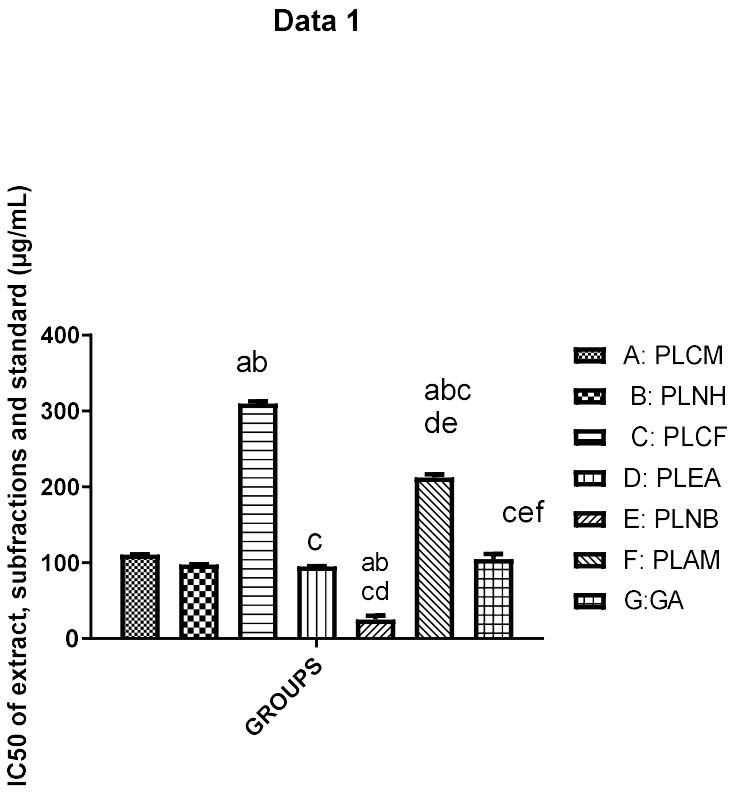


Figure 23: Showing the IC 50 values of *P. americana* leaf extract and fractions and gallic acid in DPPH radical scavenging assay

Group A: *P. americana* leaf crude methanol extract (PLCM) Group B: *P.americana* leaf N-Hexane fraction (PLNH). Group C: *P.americana* leaf Chloroform fraction (PLCF), Group D :*P.americana* leaf Ethylacetate fraction (PLEA), Group E : *P.americana* leaf N-Butanol fraction (PLNB), Group F : *P.americana* leaf Aqueous Methanol fraction (PLAM), Group G : Gallic acid (GA). Significance at $\alpha_{0.05}$

NITRIC OXIDE RADICAL SCAVENGING ASSAY OF P. A. LEAF EXTRACT AND FRACTION.

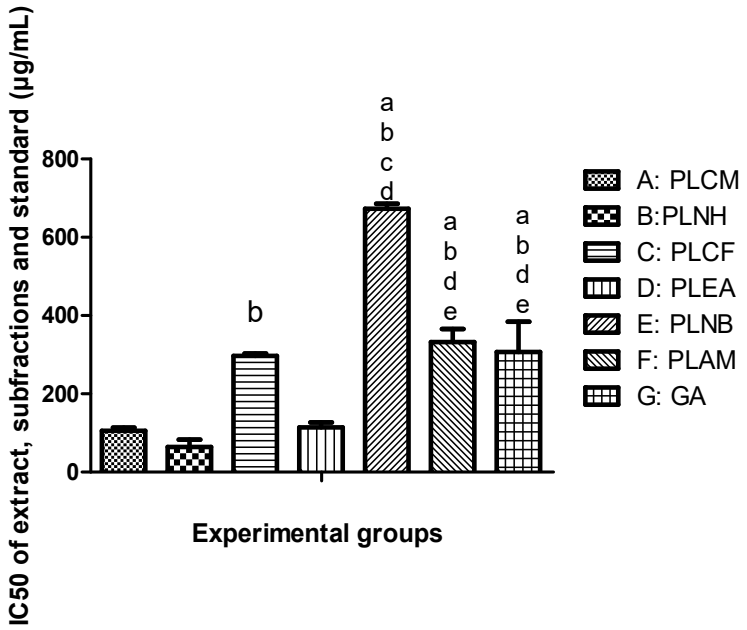


Figure 24: Showing the IC 50 values of *P. americana* leaf extract and fractions and gallic acid in Nitric oxide radical scavenging assay

Group A: *P. americana* leaf crude methanol extract (PLCM) Group B: *P.americana* leaf N-Hexane fraction (PLNH), Group C :*P.americana* leaf Chloroform fraction (PLCF), Group D : *P.americana* leaf Ethylacetate fraction (PLEA), Group E : *P.americana* leaf N-Butanol fraction (PLNB), Group F : *P.americana* leaf Aqueous Methanol fraction (PLAM), Group G : Gallic acid (GA). Significance at $\alpha_{0.05}$

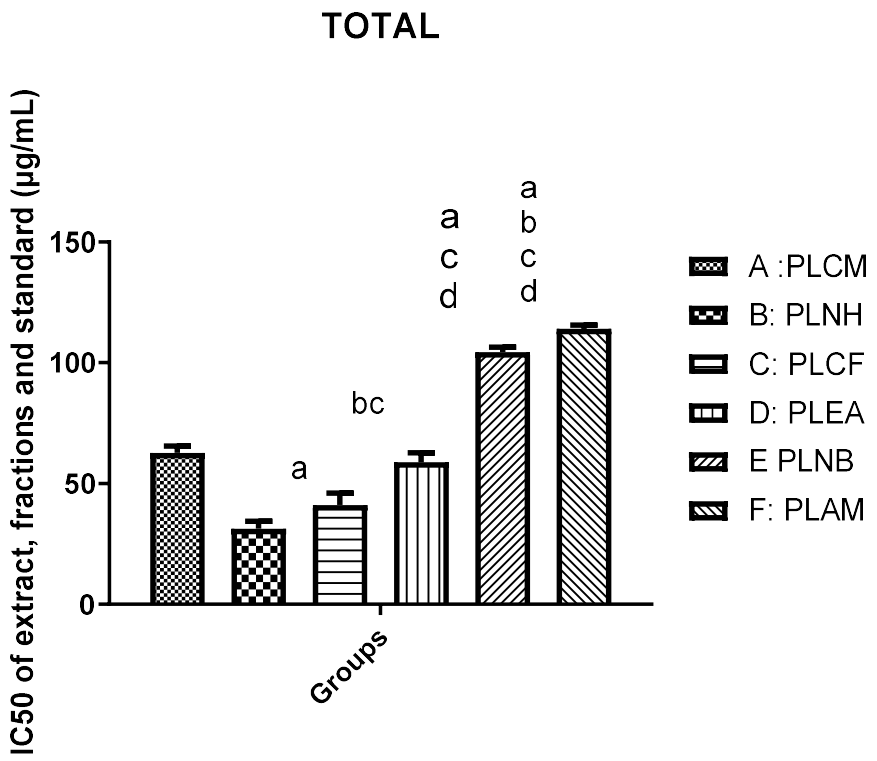


Figure 25: Showing the total phenolic content of *P. americana* leaf extract and fractions and gallic acid in Nitric oxide radical scavenging assay

Group A: *P. americana* leaf crude methanol extract (PLCM) Group B: *P.americana* leaf N-Hexane fraction (PLNH), Group C:*P.americana* leaf Chloroform fraction (PLCF), Group D : *P.americana* leaf Ethylacetate fraction (PLEA), Group E : *P.americana* leaf N-Butanol fraction (PLNB), Group F : *P.americana* leaf Aqueous Methanol fraction (PLAM), Group G : Gallic acid (GA). Significance at $\alpha_{0.05}$

Anti-inflammatory assay of the *Persea americana* leaf crude methanol extract, fractions and standard antiimflammatory drug.

Table 1: Showing the IC 50 values of P. americana leaf extract and fractions and Quercetin and indomethacin in anti inflammatory assay.

Extracts, fractions& standards	Mean ± SEM
A: PLCM	191.53 ±15.2
B: PLNH	100.24 ± 7.63^{a}
C: PLCF	106.42 ± 0.81^{a}
D: PLEA	100.86 ± 2.77^{a}
E: PLNB	75.36 ± 4.03^{a}
F: PLAM	96.65 ± 8.12^{a}
G: Quercetin	59.33 ± 5.4^{abcdf}
H: Indomethacine	115.09 ± 1.62^{aeg}

Group A: *P. americana* leaf crude methanol extract (PLCM) Group B: *P.americana* leaf N-Hexane fraction (PLNH), Group C: *P.americana* leaf Chloroform fraction (PLCF), Group D: *P.americana* leaf Ethylacetate fraction (PLEA), Group E: *P.americana* leaf N-Butanol fraction (PLNB), Group F: *P.americana* leaf Aqueuos methanol fraction (PLAM), Group G: Gallic acid (GA). Significance at $\alpha_{0.05}$

METAL CHELATING ASSAY OF P. AMERICANA BARK EXTRACT AND FRACTIONS

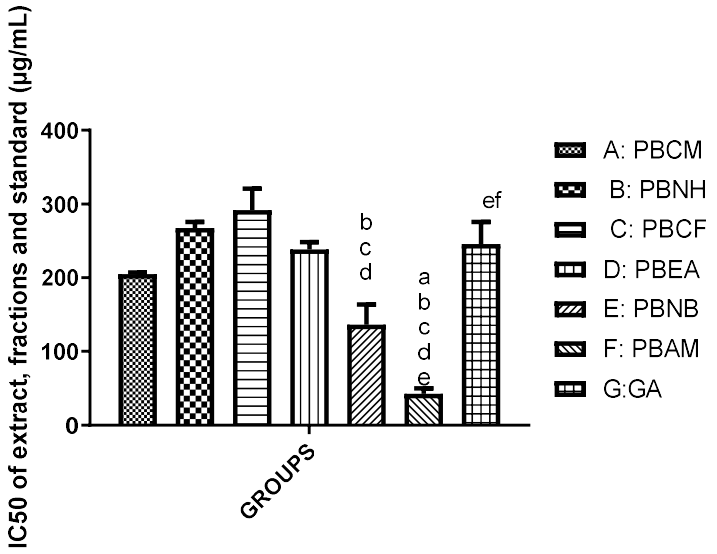


Figure 26: The IC 50 values of *P. americana* bark extract and fractions and gallic acid in metal chelating assay

Group A: *P. americana* leaf crude methanol extract (PBCM) Group B: *P.americana* leaf N-Hexane fraction (PBNH), Group C: *P.americana* leaf Chloroform fraction (PBCF), Group D: *P.americana* leaf Ethylacetate fraction (PBEA), Group E: *P.americana* leaf N-Butanol fraction (PBNB), Group F: *P.americana* leaf Aqueuos methanol fraction (PBAM), Group G: Gallic acid (GA). Significance at $\alpha_{0.05}$

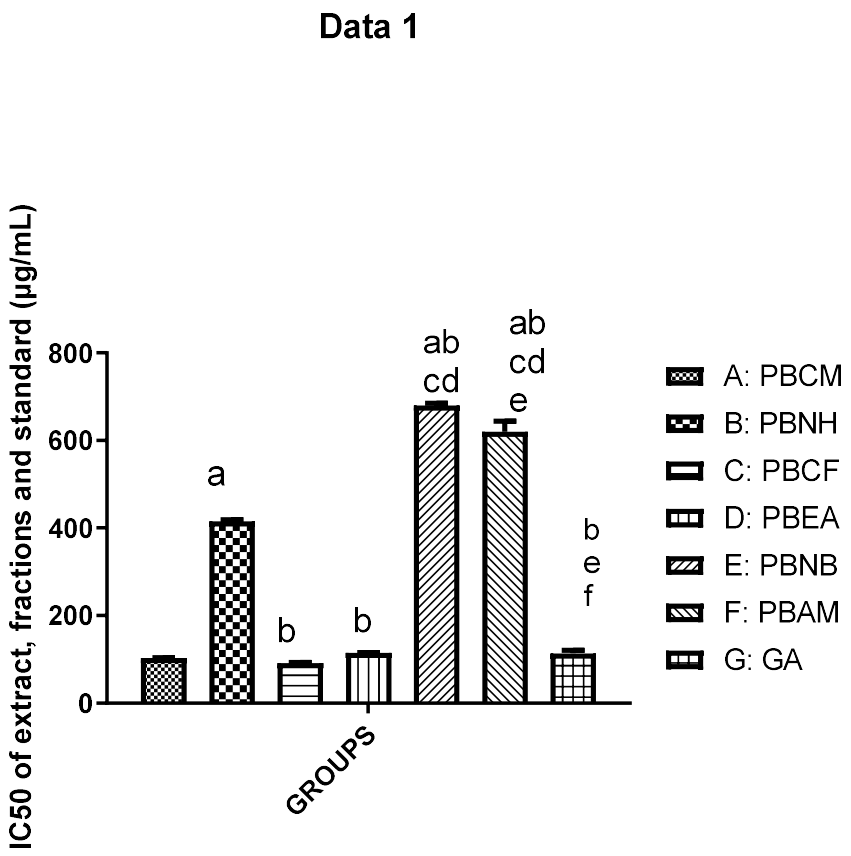


Figure 27: Showing the IC 50 values of *P. americana* bark extract and fractions and gallic acid in DPPH assay

Group A: *P. americana* leaf crude methanol extract (PBCM) Group B: *P.americana* leaf N-Hexane fraction (PBNH), Group C: *P.americana* leaf Chloroform fraction (PBCF), Group D: *P.americana* leaf Ethylacetate fraction (PBEA), Group E: *P.americana* leaf N-Butanol fraction (PBNB), Group F: *P.americana* leaf Aqueuos methanol fraction (PBAM), Group G: Gallic acid (GA). Significance at $\alpha_{0.05}$

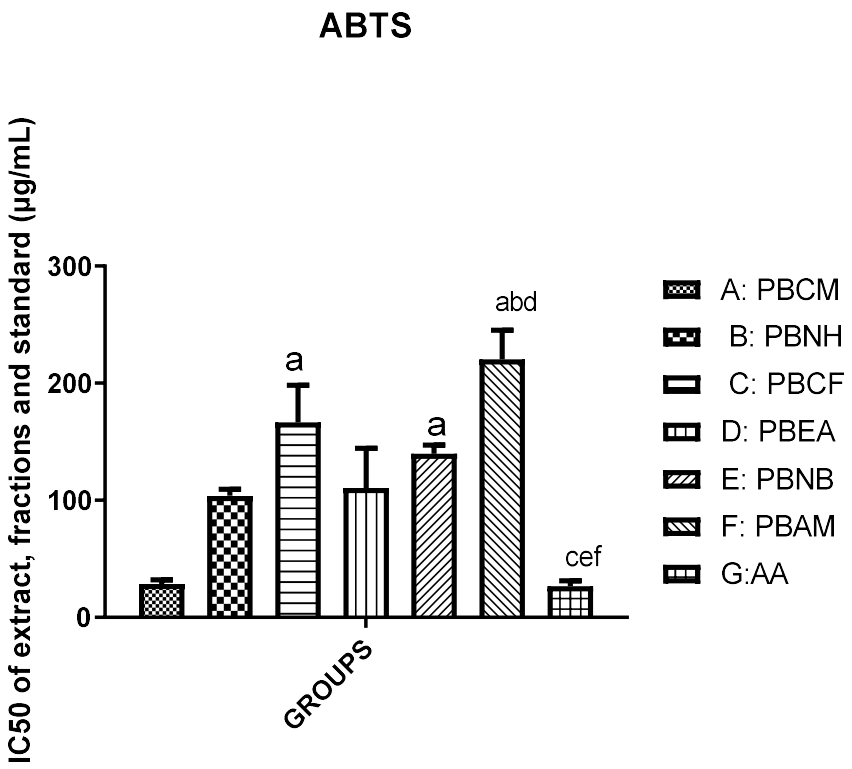


Figure 28: Showing the IC 50 values of *P. americana* bark extract and fractions and gallic acid in ABTS assay

Group A: *P. americana* leaf crude methanol extract (PBCM) Group B: *P.americana* leaf N-Hexane fraction (PBNH), Group C: *P.americana* leaf Chloroform fraction (PBCF), Group D: *P.americana* leaf Ethylacetate fraction (PBEA), Group E: *P.americana* leaf N-Butanol fraction (PBNB), Group F: *P.americana* leaf Aqueuos methanol fraction (PBAM), Group G: Gallic acid (GA). Significance at $\alpha_{0.05}$

NITRIC OXIDE ASSAY.

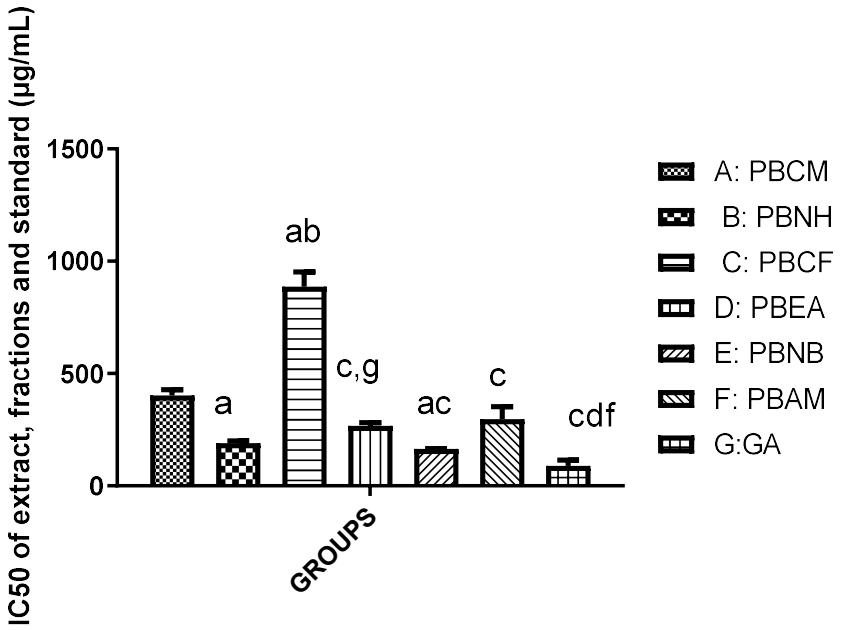


Figure 29: Showing the IC 50 values of *P. americana* bark extract and fractions and gallic acid in nitric oxide assay

Group A: *P. americana* leaf crude methanol extract (PBCM) Group B: *P.americana* leaf N-Hexane fraction (PBNH), Group C: *P.americana* leaf Chloroform fraction (PBCF), Group D: *P.americana* leaf Ethylacetate fraction (PBEA), Group E: *P.americana* leaf N-Butanol fraction (PBNB), Group F: *P.americana* leaf Aqueuos methanol fraction (PBAM), Group G: Gallic acid (GA). Significance at $\alpha_{0.05}$

HYDROXYL RADICAL SCAVENGING ASSAY

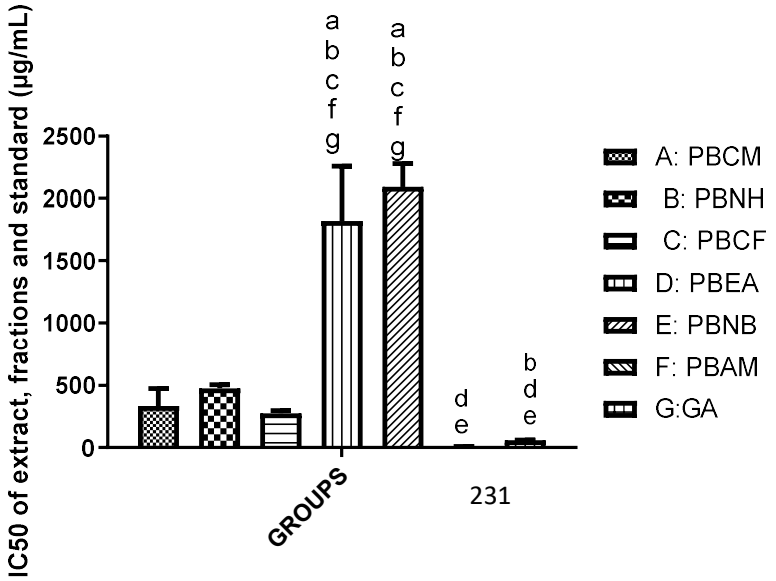


Figure 30: Showing the IC 50 values of *P. americana* bark extract and fractions and gallic acid in Hydroxyl radical scavenging assay.

Group A: *P. americana* leaf crude methanol extract (PBCM) Group B: *P.americana* leaf N-Hexane fraction (PBNH), Group C: *P.americana* leaf Chloroform fraction (PBCF), Group D: *P.americana* leaf Ethylacetate fraction (PBEA), Group E: *P.americana* leaf N-Butanol fraction (PBNB), Group F: *P.americana* leaf Aqueous methanol fraction (PBAM), Group G: Gallic acid (GA). Significance at $\alpha_{0.05}$

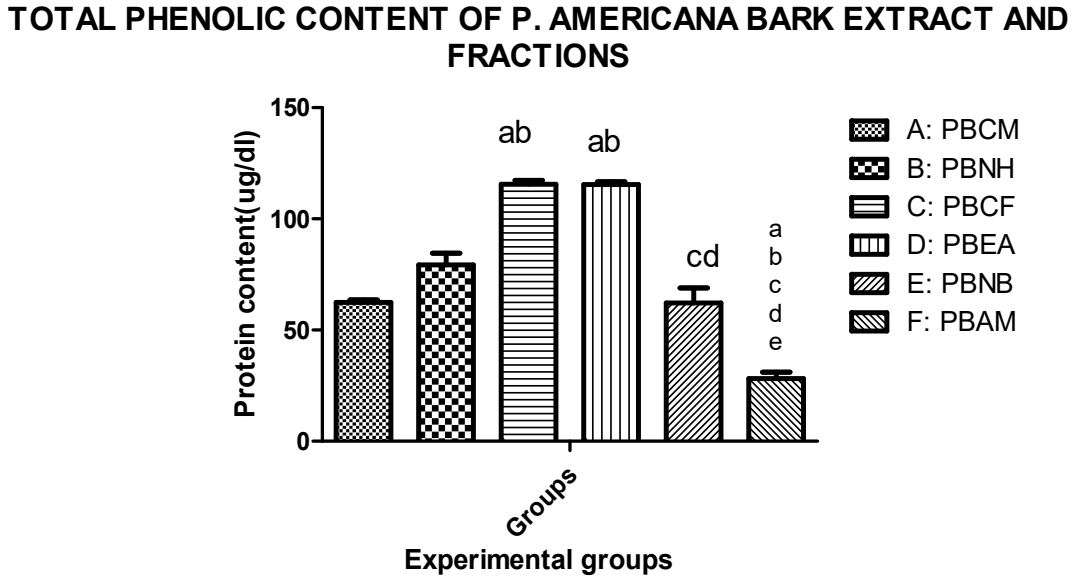


Figure 31: Showing the total phenolic content of the *P. americana* bark extract and fractions. Group A: *P. americana* root crude methanol extract (PRCM) Group B: *P.americana* root N-Hexane fraction (PRNH), Group C: *P.americana* root

Chloroform fraction (PRCF), Group D: *P.americana* root Ethylacetate fraction (PREA), Group E: *P.americana* root N-Butanol fraction (PRNB), Group F: *P.americana* root Aqueuos methanol fraction (PRAM), Group G: Gallic acid (GA). Significance at $\alpha_{0.05.}$ Superscripts a - g show that the groups were significant when compared with groups A - G respectively.

Table 2: Showing the IC 50 values of *P. americana* root extract, fractions, Quercetin and Indomethacine in Anti- inflammatory assay

Extracts, Fractions & Standards	Mean ± SEM
A: PRCM	101.51±9.01
B: PRNH	69.17 ± 3.9^{a}
C: PRCF	101.84 ± 8.97^{b}
D: PREA	36.98 ± 7.14^{abc}
E:PRNB	119.56 ± 13.9^{bd}
F: PRAM	99.6 ± 6.04^{d}
G: Quercetin	59.33 ± 5.4^{acef}
H: Indomethacin	115.09 ± 1.62^{bdg}

Group A: *P. americana* leaf crude methanol extract (PLCM) Group B: *P.americana* leaf N-Hexane fraction (PLNH), Group C: *P.americana* leaf Chloroform fraction (PLCF), Group D: *P.americana* leaf Ethylacetate fraction (PLEA), Group E: *P.americana* leaf N-Butanol fraction (PLNB), Group F: *P.americana* leaf Aqueuos methanol fraction (PLAM), Group G: Gallic acid (GA). Significance at $\alpha_{0.05}$

METAL CHELATING FOR ASSAY P. AMERICANA ROOT EXTACTS AND FRACTIONS

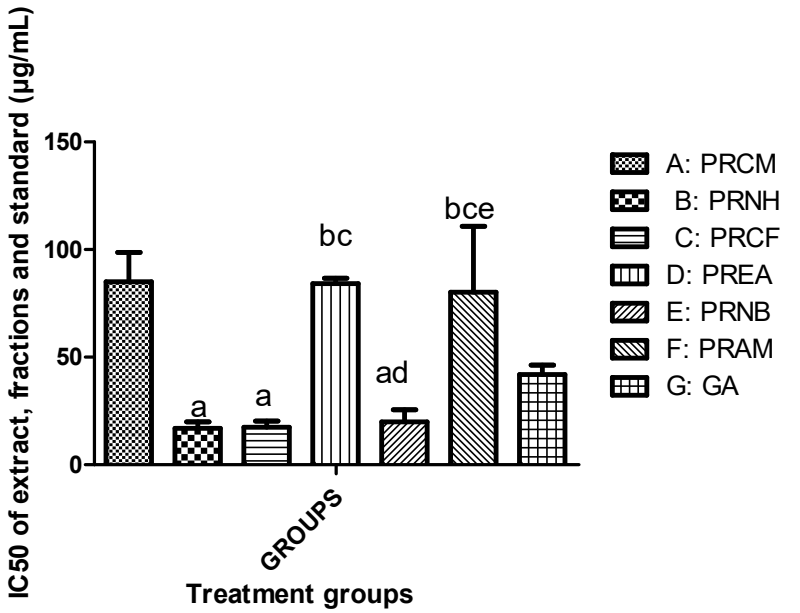


Figure 32: Showing the IC 50 values of *P. americana* root extract, fractions and gallic acid in metal chelating assay

Group A: *P. americana* root crude methanol extract (PRCM) Group B: *P.americana* root N-Hexane fraction (PRNH), Group C: *P.americana* root Chloroform fraction (PRCF), Group D: *P.americana* root Ethylacetate fraction (PREA), Group E: *P.americana* root N-Butanol fraction (PRNB), Group F: *P.americana* root Aqueuos methanol fraction (PRAM), Group G: Gallic acid (GA). Significance at $\alpha_{0.05.}$ Superscripts a - g show that the groups were significant when compared with groups A - G respectively.

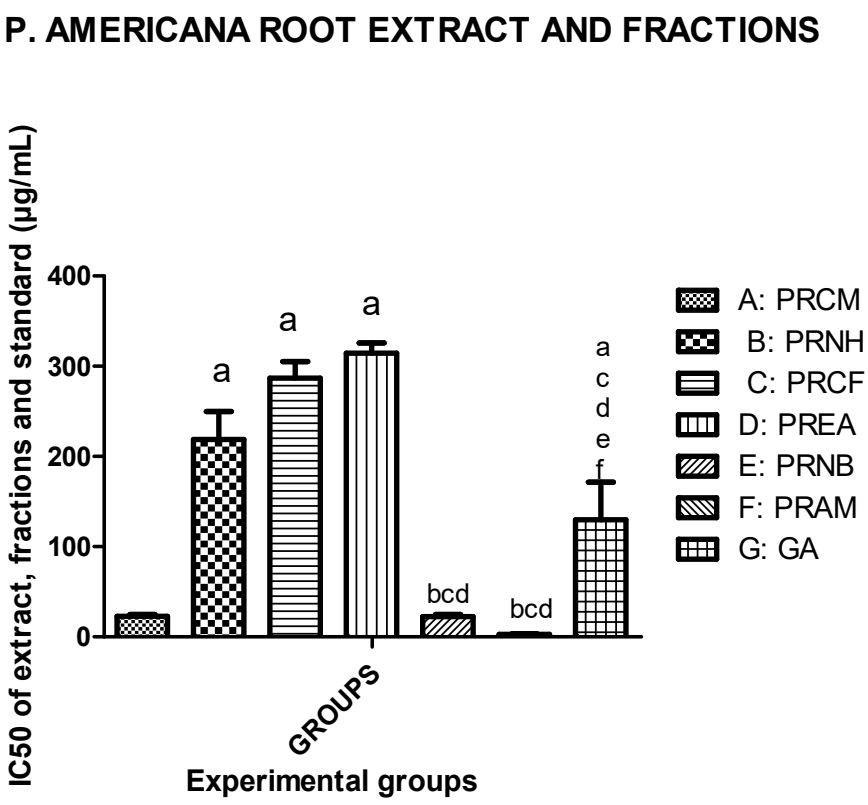


Figure 33:Showing the IC 50 values of *P. americana* root extract, fractions and gallic acid in ABTS Assay

Group A: *P. americana* root crude methanol extract (PRCM) Group B: *P.americana* root N-Hexane fraction (PRNH), Group C: *P.americana* root Chloroform fraction (PRCF), Group D: *P.americana* root Ethylacetate fraction (PREA), Group E: *P.americana* root N-Butanol fraction (PRNB), Group F: *P.americana* root Aqueous methanol fraction (PRAM), Group G: Gallic acid (GA). Significance at $\alpha_{0.05.}$ Superscripts a - g show that the group were significant when compared with groups A - G respectively.

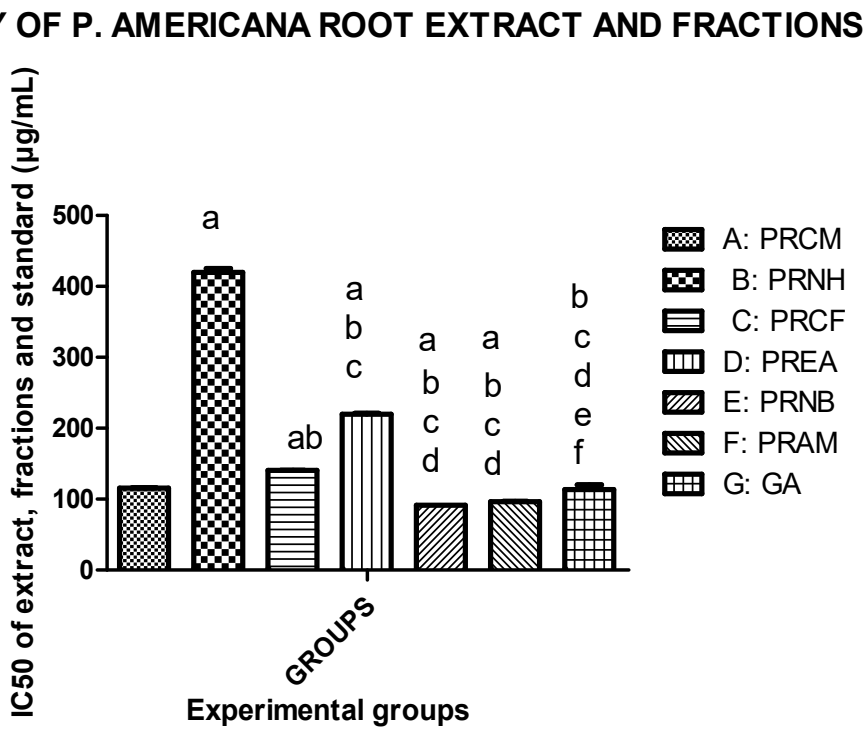


Figure 34: The IC 50 values of *P. americana* root extract, fractions and gallic acid in DPPH assay

Group A: *P. americana* root crude methanol extract (PRCM) Group B: *P.americana* root N-Hexane fraction (PRNH), Group C: *P.americana* root Chloroform fraction (PRCF), Group D: *P.americana* root Ethylacetate fraction (PREA), Group E: *P.americana* root N-Butanol fraction (PRNB), Group F: *P.americana* root Aqueuos methanol fraction (PRAM), Group G: Gallic acid (GA). Significance at $\alpha_{0.05.}$ Superscripts a - g show that the groups were significant when compared with groups A - G respectively.

HYDROXYL INVITRO ASSAY OF P. AMERICANA ROOT EXTRACT AND FRACTIONS

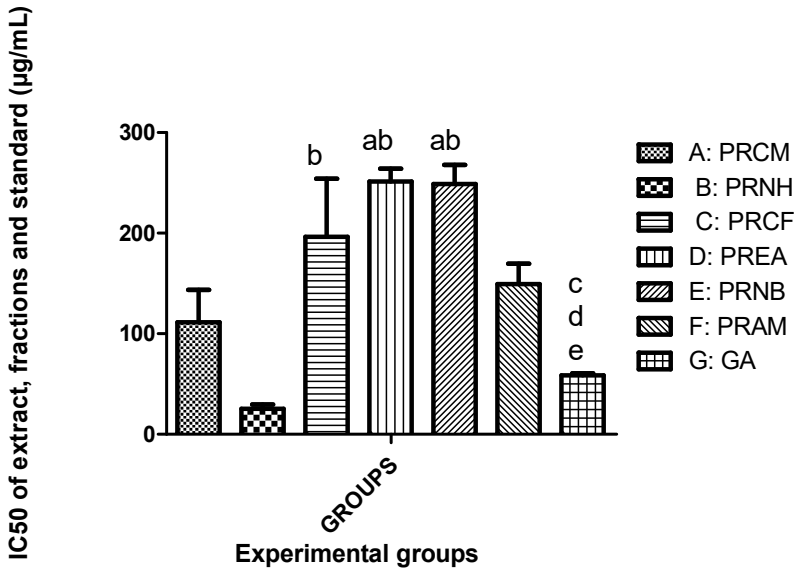


Figure 35:The IC 50 values of *P. americana* root extract, fractions and gallic acid in Hydroxyl radical scavenging assay.

Group A: *P. americana* root crude methanol extract (PRCM) Group B: *P.americana* root N-Hexane fraction (PRNH), Group C: *P.americana* root Chloroform fraction (PRCF), Group D: *P.americana* root Ethylacetate fraction (PREA), Group E: *P.americana* root N-Butanol fraction (PRNB), Group F: *P.americana* root Aqueuos methanol fraction (PRAM), Group G: Gallic acid (GA).Superscripsts a - g show that the groups were significant when compared with groups A - G respectively. Significance at $\alpha_{0.05..}$

N. OXIDE ASSAY OF P. AMERICANA ROOT EXTRACT AND FRACTIONS

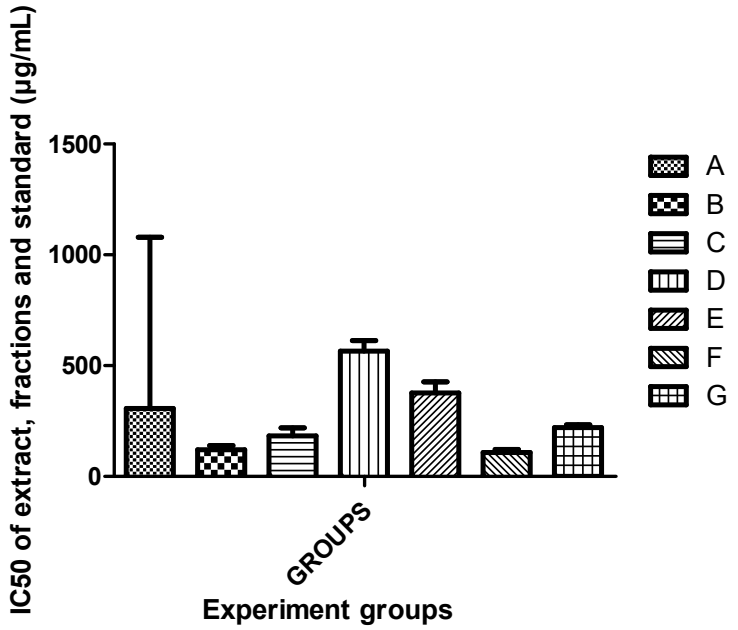


Figure 37:The IC 50 values of *P. americana* root extract, fractions and gallic acid in Nitric Oxide scarvenging assay

Group A: *P. americana* root crude methanol extract (PRCM) Group B: *P.americana* root N-Hexane fraction (PRNH), Group C: *P.americana* root Chloroform fraction (PRCF), Group D: *P.americana* root Ethylacetate fraction (PREA), Group E: *P.americana* root N-Butanol fraction (PRNB), Group F: *P.americana* root Aqueuos methanol fraction (PRAM), Group G: Gallic acid (GA). Superscripsts a - g show that the groups were significant when compared with groups A - G respectively. Significance at $\alpha_{0.05.}$

TOTAL PHENOLIC CONTENT OF P. AMERICANA ROOT EXTRACT AND FRACTION

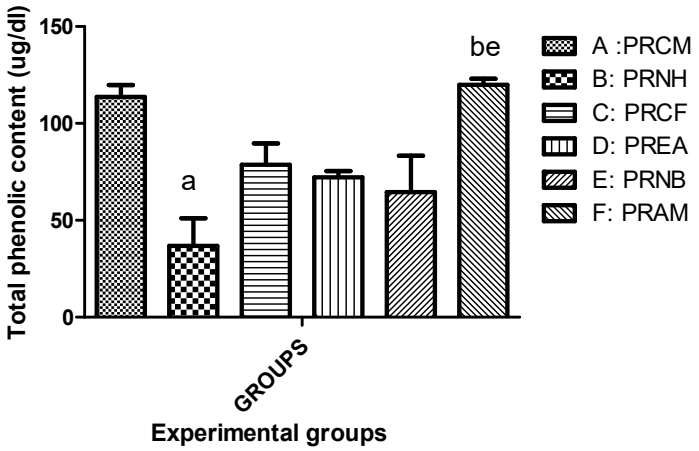


Figure 38: The Total phenolic content of the *P. americana* root extract and fractions.

Group A: *P. americana* root crude methanol extract (PRCM) Group B: *P.americana* root N-Hexane fraction (PRNH), Group C: *P.americana* root Chloroform fraction (PRCF), Group D: *P.americana* root Ethylacetate fraction (PREA), Group E: *P.americana* root N-Butanol fraction (PRNB), Group F: *P.americana* root Aqueuos methanol fraction (PRAM). Superscripts a,b, e shows that the group was significant when compared with groups A, B, and E respectively. Significance at $\alpha_{0.05}$

Table 3: The IC 50 values of *P. americana* root extract, fractions, Quercetin and Indomethacine in Anti- inflammatory assay

Extracts, Fractions & Standards	Mean ± SEM
A: PRCM	101.74 ±13.1
B: PRNH	101.82±6.62
C: PRCF	102.85±9.47
D: PREA	64.72 ± 8.14^{abc}
E:PRNB	104.76 ± 0.93^{d}
F: PRAM	101.54 ± 5.52^{d}
G: Quercetin	59.33 ± 5.4^{abcef}
H: Indomethacin	115.09 ± 1.62^{dg}

Group A: *P. americana* leaf crude methanol extract (PLCM) Group B: *P.americana* leaf N-Hexane fraction (PLNH), Group C: *P.americana* leaf Chloroform fraction (PLCF), Group D: *P.americana* leaf Ethylacetate fraction (PLEA), Group E: *P.americana* leaf N-Butanol fraction (PLNB), Group F: *P.americana* leaf Aqueuos methanol fraction (PLAM), Group G: Gallic acid (GA). Significance at $\alpha_{0.05}$

Table 4: The summary of the biological activities of the *P. americana*leaf extracts.

	METAL. CHELATING	ABTS	Hydroxyl Radical Scarvenging	DPPH	Nitric Oxide	REDUCING POWER	Anti-Inflammation Assay
PLCM	xxx	xx	x	xx	xxx	-	-
PLNH	xx	xxx	-	xx	xxx	-	xx
PLCF	xx	xx	xxx	-	xx	-	xx
PLEA	xx	xxx	x	xx	xxx	xx	xx
PLNB	xx	xxxx	-	xxx	-	xx	xx
PLAM	xx	-	xxx	-	xx	x	xx
GA	xx	xx	xx	xx	xx	xx	xx

Table 5: The summary of the biological activities of the *P. americana* bark extracts.

	Metal Chalating	**ABTS**	**Hydroxyl Radical**	**DPPH**	**Nitric Oxide**	**Reducing Power**	**Anti-Inflammation**
PBCM	xx	xx	x	xx	x	x	xx
PBNH	x	x	x	-	x	x	xxx
PBCF	-	-	x	xx	-	xx	xx
PBEA	xx	x	-	xx	-	xx	xx
PBNB	xxx	x	-	-	xx	xx	xxxx
PBAM	xxx	-	xxx	-	x	-	xx
GA	xx	xx	xx	xx	xx	xxx	xx

Table 6: The summary of the biological activities of the *P. americana*root extracts.

	Metal Chelating	ABTS	Nitric Oxide	DPPH	Hydroxyl Radical	Reducing Power	Anti-Inflammation
PRCM	-	xxx	xx	xx	x	-	xx
PRNH	XXX	-	xxx	-	xxx	-	xx
PRCF	XXX	-	xx	xx	-	xx	xx
PREA	-	-	x	-	-	xxx	xxxx
PRNB	XXX	xxx	x	xxx		x	xx
PRAM	-	xxx	xxx	xxx	-	xx	xx
GA	XX	xx	xx	xx	xx	xx	xx

Table 7: The biological antioxidant activities of pure metabolytes of *P. americana* leaf and root

PURE COMPOUNDS	MEAN±STANDARD ERROR OF MEAN				
	ABTS ASSAY	**DPPH ASSAY**	**HYDROXYL ASSAY**	**METAL CHELATING ASSAY**	**N.OXIDE ASSAY**
A:RBC1	15.20±2.55	128.77±6.43	156.09±12.56	99.29±7.87	103.07±9.56
B:RCACA	3.47±1.31	43.33±10.00^{a}	15.65±2.10^{a}	100.81±15.86	223.43±47.34^{a}
C:REACB	49.68±8.90^{b}	134.70±6.38^{b}	3.27±0.12^{a}	84.85±14.49	67.81±6.82^{b}
D:REACC	7.83±0.44^{c}	30.81±0.80^{c}	14.29±0.39^{a}	9.55±3.41	62.51±9.91^{b}
E:REACD	61.79±15.47^{abd}	319.00±12.63^{abcd}	76.75±8.95^{ac}	249.27±2.7^{3abcd}	151.58±8.21^{cd}
F:REACE	50.66±3.18^{bd}	261.708±11.49^{abcde}	8.35±1.03^{ae}	79.13±8.05^{e}	83.09±19.74^{b}
G:REACF	50.22±1.81^{bd}	6.44±0.61^{cdef}	91.49±1.81^{abcdf}	173.44±62.25^{d}	91.13±3.90^{b}
H:LEACA	16.81±1.00^{e}	299.98±20.40^{abcdg}	133.81±11.27^{bcdf}	71.98±1.61^{e}	4.49±3.99^{abeg}
I:LEACB	18.99±6.01^{e}	22.45±1.59^{acefgh}	47.72±26.94^{ah}	276.87±30.27^{adcdfh}	87.86±9.95^{b}
J:LEACC	118.76±13.29^{a} ^{bcdefghi}	44.39±1.16^{acefh}	3.28±0.81^{aegh}	477.95±19.82^{abcdefghı}	116.04±0.93^{bh}
K:LEACD	13.21±3.26^{efgj}	162.70±12.49^{bdefgh}	260.19±27.33^{abc} ^{defghij}	168.25±11.24^{dj}	46.22±10.80^{be}
L:GA	16.12±7.71^{ej}	31.23±2.78^{acefghk}	44.46±2.85^{ahk}	217.48±11.56^{abcdfhı}	61.29±7.53^{be}

Group A: RBCA, Group B: REACA, Group C: REACB, Group D: REACC, Group E: REACD, Group F: REACE, Group G: REACF, Group H: LEACA Group I: LEACB, Group J: LEACC, Group K: LEACD, Group L: Gallic acid (GA). Superscripsts a - l show that the groups were significant when compared with groups A - L respectively. Significance at $\alpha_{0.05}$

Table 8: The summary of the biological activities of the pure compounds of *P. americana* extracts.

	MC	ABTS	H RADICAL	DPPH	NO
RBCA	xxx	xx	x	x	x
REACA	xxx	xxx	xxx	xx	-----
REACB	xxx	x	xxx	x	xx
REACC	xxxx	xxx	xxx	xx	xx
REACD	x	x	x		-----
REACE	xxx	x	xxx		x
REACF	x	x	x	x	x
LEACA	xxx	xx	-----	------	xxx
LEACB	------	xx	x	xx	x
LEACC	-------	------	xxx	xx	x
LEACD	xxx	xx	------	x	xxx
GA	xx	xx	xx	xx	xx

NMR SPECTRA OF THE PURE COMPOUNDS

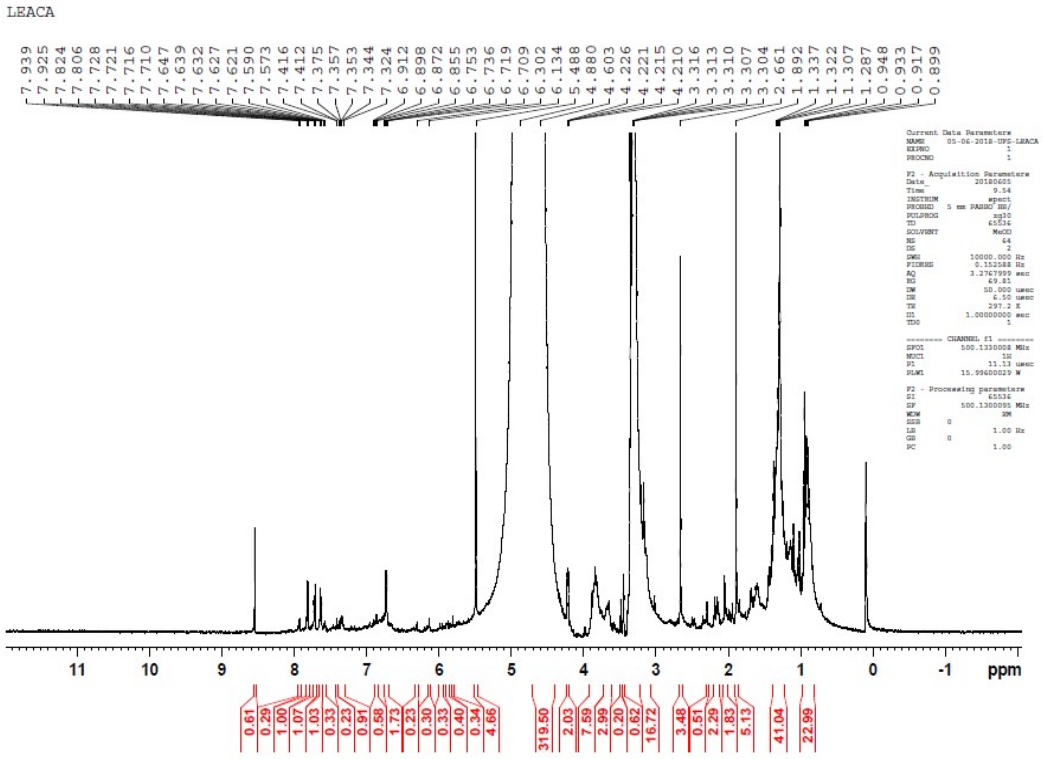


Figure 39: COMPOUND A OF THE ETHYL ACETATE EXTRACT OF THE PERSIA AMERICANA LEAVE

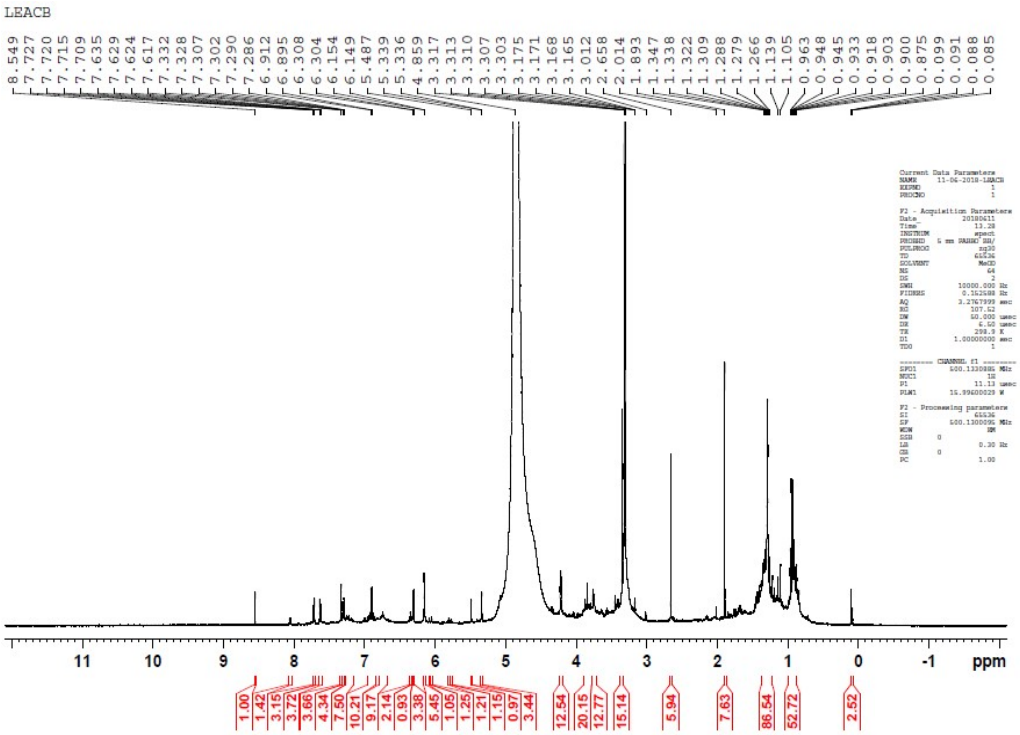


Figure 40: COMPOUND B OF THE ETHYL ACETATE EXTRACT OF THE PERSIA AMERICANA LEAVE

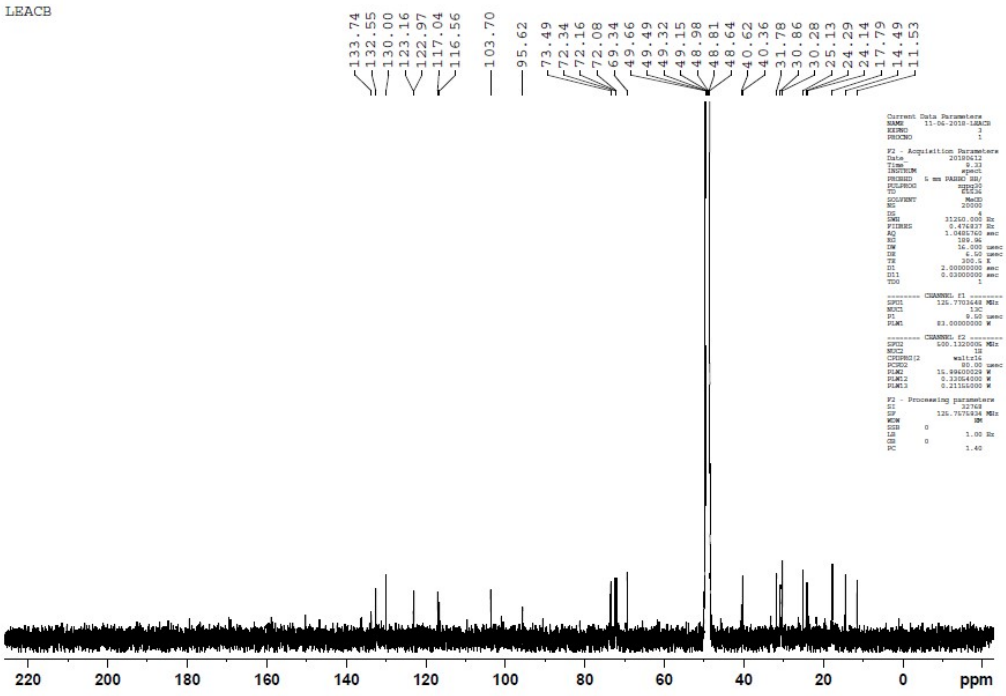


Figure 41: NMR 13 C FOR THE COMPOUND B OF THE ETHYL ACETATE EXTRACT OF THE PERSIA AMERICANA LEAVE

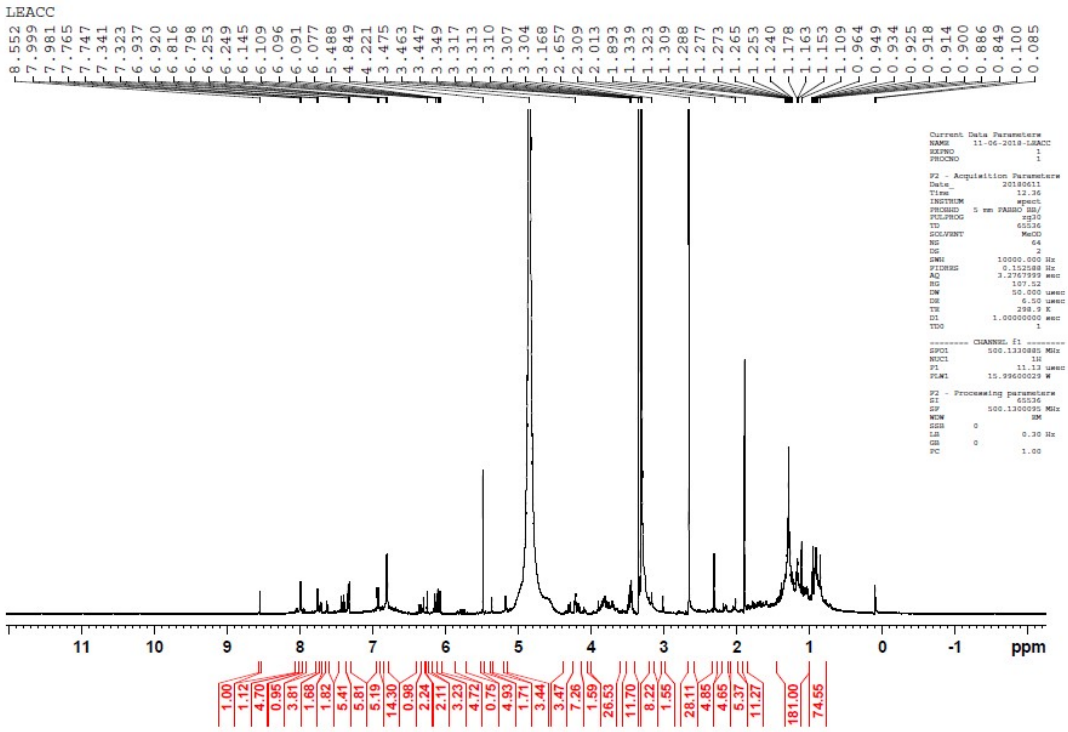


Figure 42: NMR 1H FOR THE COMPOUND C OF THE ETHYL ACETATE EXTRACT OF THE PERSIA AMERICANA LEAF

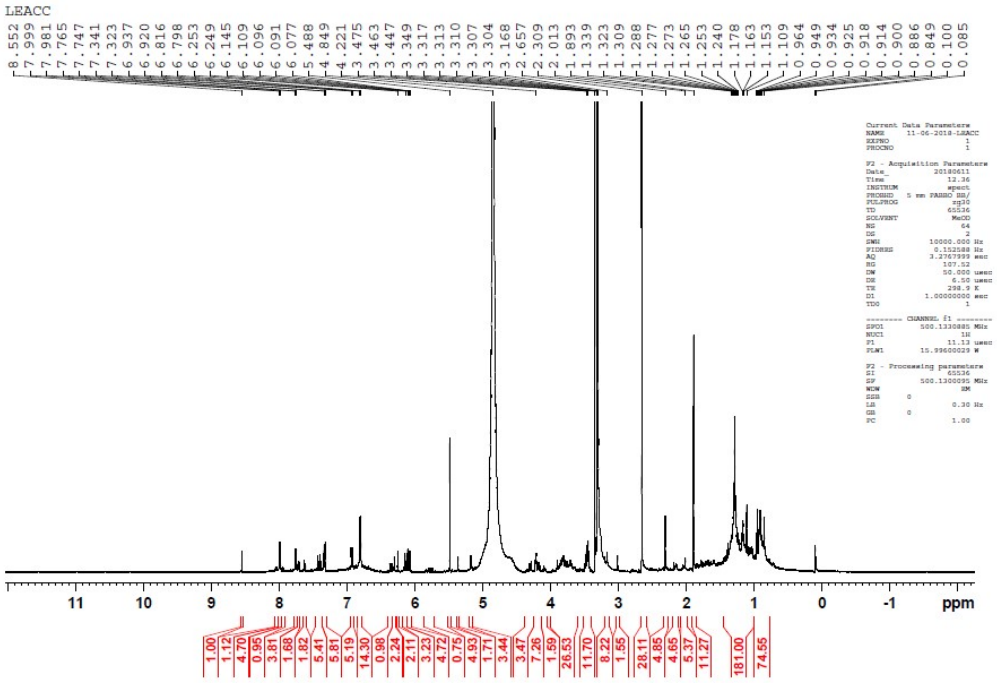


Figure 43: NMR 1H FOR THE COMPOUND C OF THE ETHYL ACETATE EXTRACT OF THE PERSIA AMERICANA LEAF

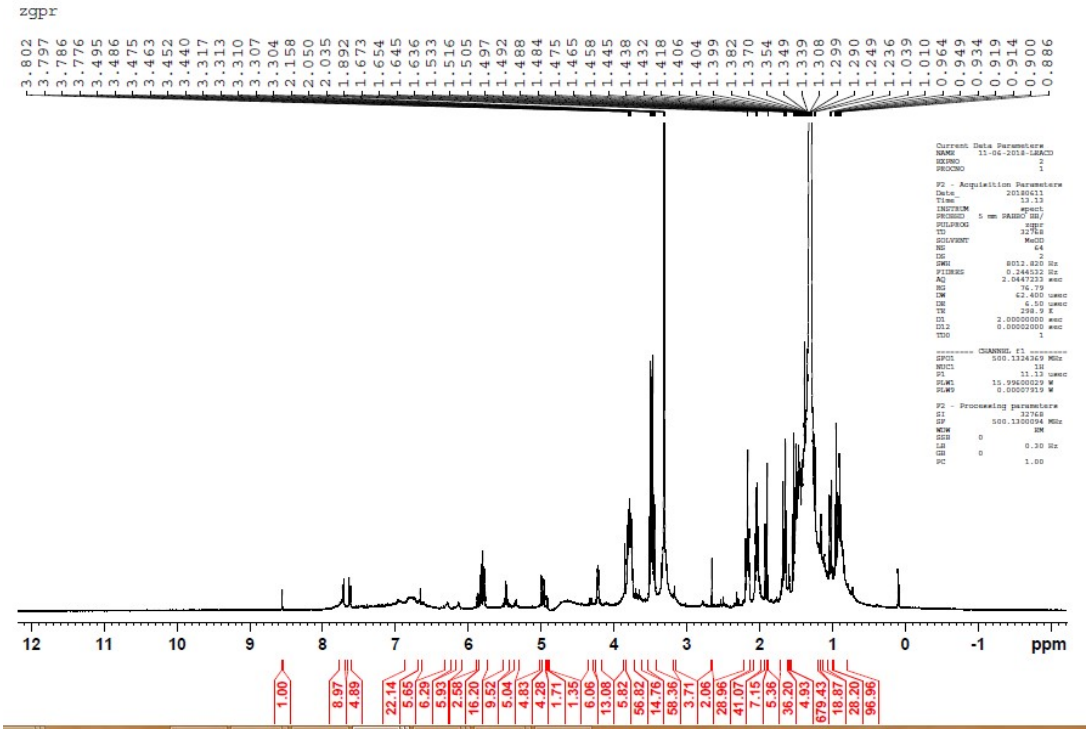


Figure 44: NMR 1H FOR THE COMPOUND D OF THE ETHYL ACETATE EXTRACT OF THE PERSIA AMERICANA LEAF

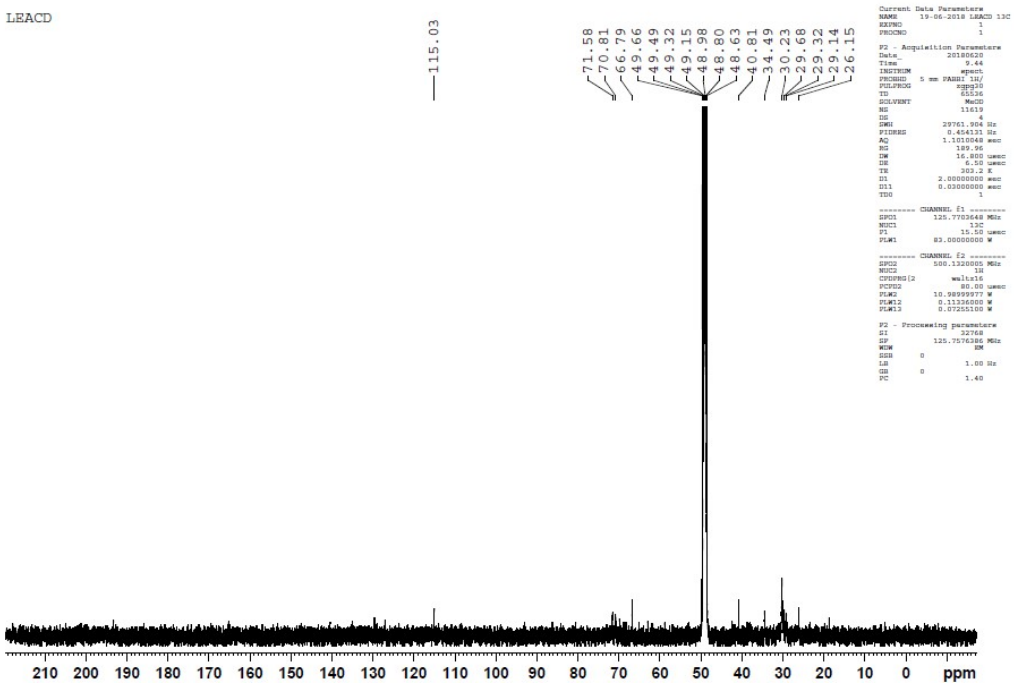


Figure 45: NMR 13C FOR THE COMPOUND D OF THE ETHYL ACETATE EXTRACT OF THE PERSIA AMERICANA LEAF.

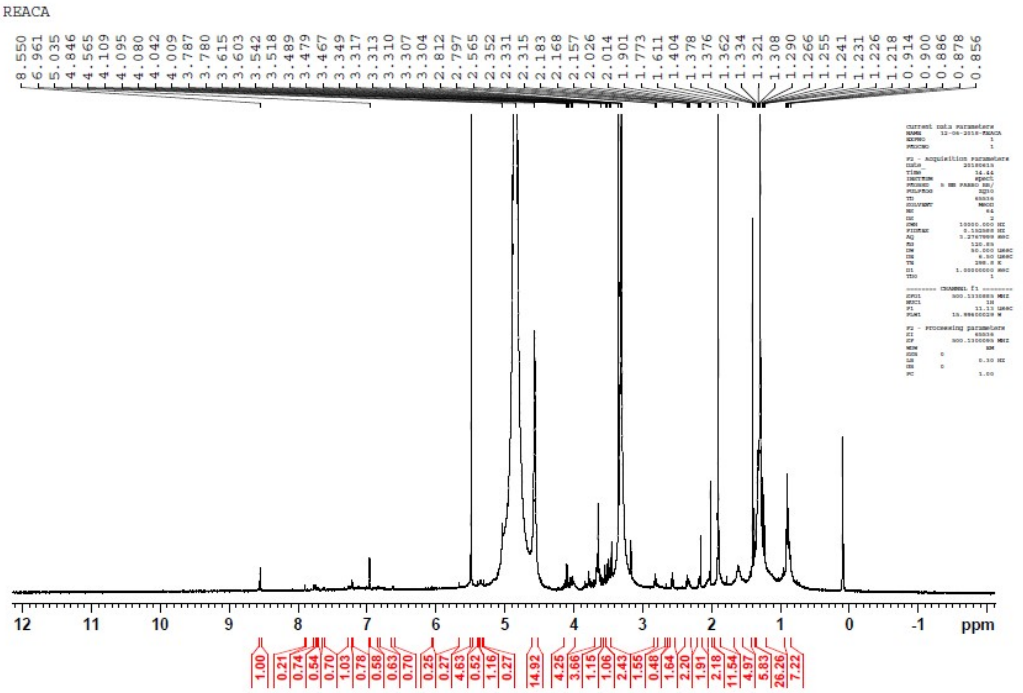


Figure 46: NMR 1H FOR THE COMPOUND A OF THE ETHYL ACETATE EXTRACT OF THE PERSIA AMERICANA ROOT.

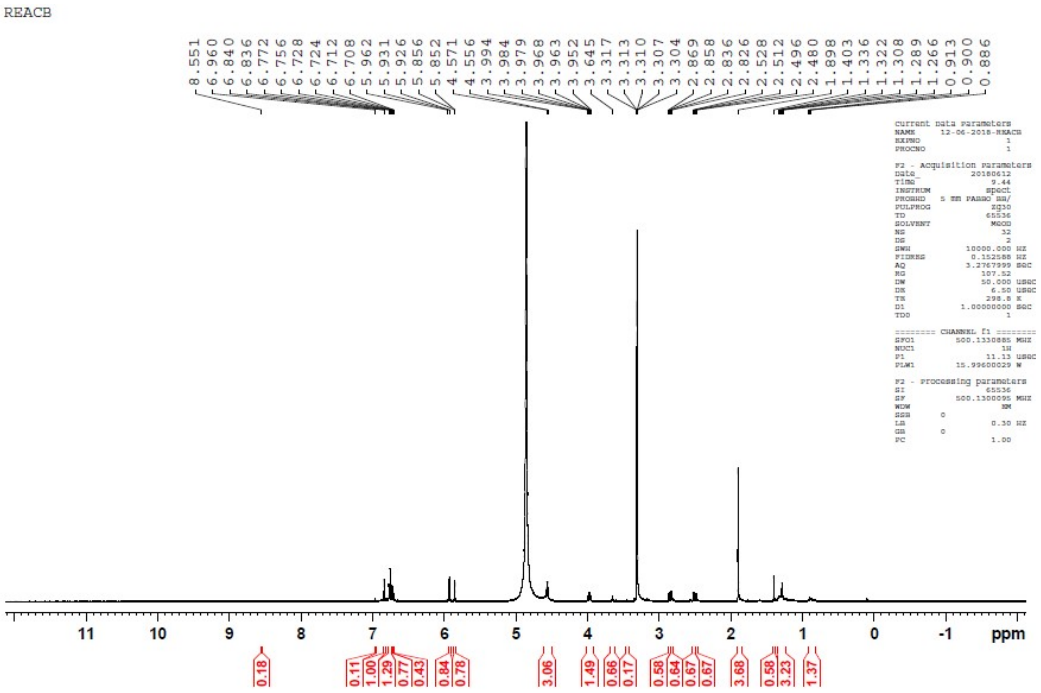


Figure 47: NMR 1H FOR THE COMPOUND B OF THE ETHYL ACETATE EXTRACT OF THE PERSIA AMERICANA ROOT

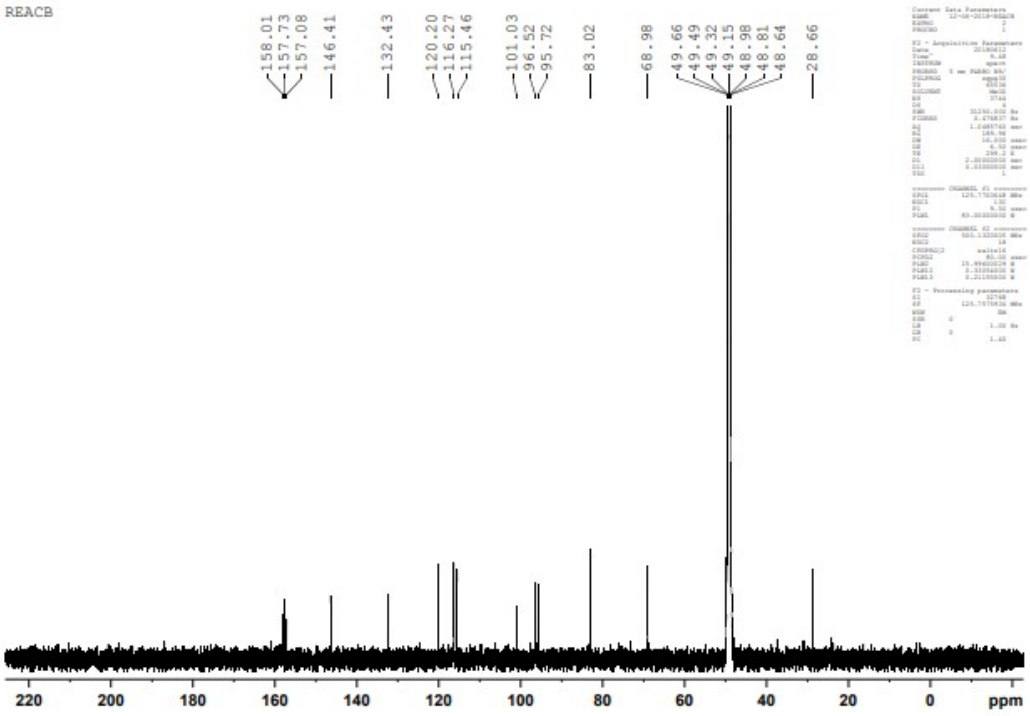


Figure 48: NMR 13 C FOR THE COMPOUND B OF THE ETHYL ACETATE EXTRACT OF THE PERSIA AMERICANA ROOT.

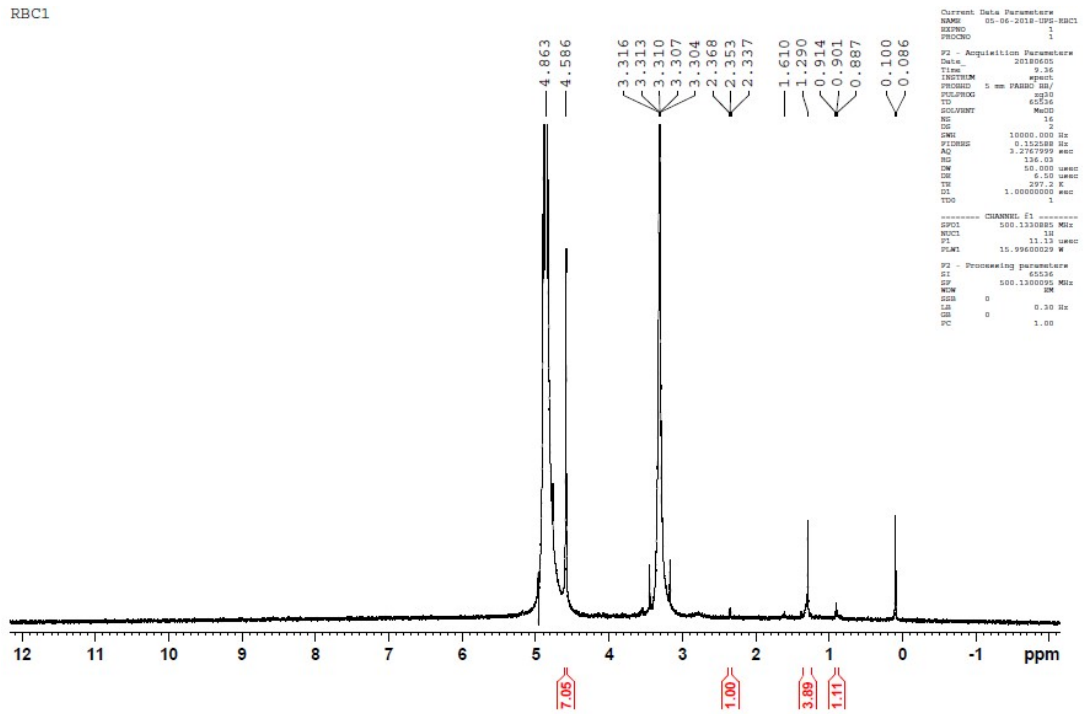


Figure 49: NMR 1H FOR THE COMPOUND A OF THE N-BUTANOL EXTRACT OF THE PERSIA AMERICANA ROOT.

FTIR SPECTRA OF THE PURE COMPOUNDS OF P.. AMERICANA PLANT

LEACA.png

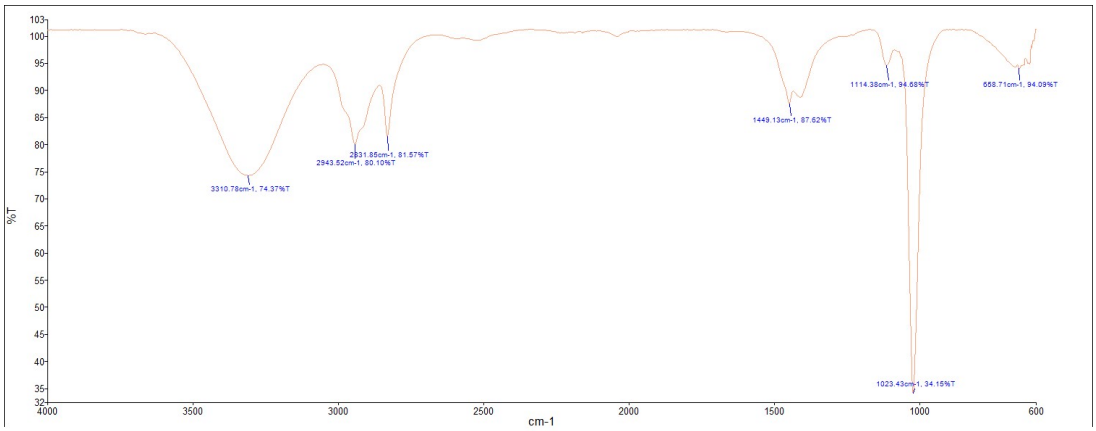


Figure 50: FTIR Spectrum of the Compound A of the Ethyl Acetate Extract (LEACA) of the *Persia americana* Leaf

LEACB.png

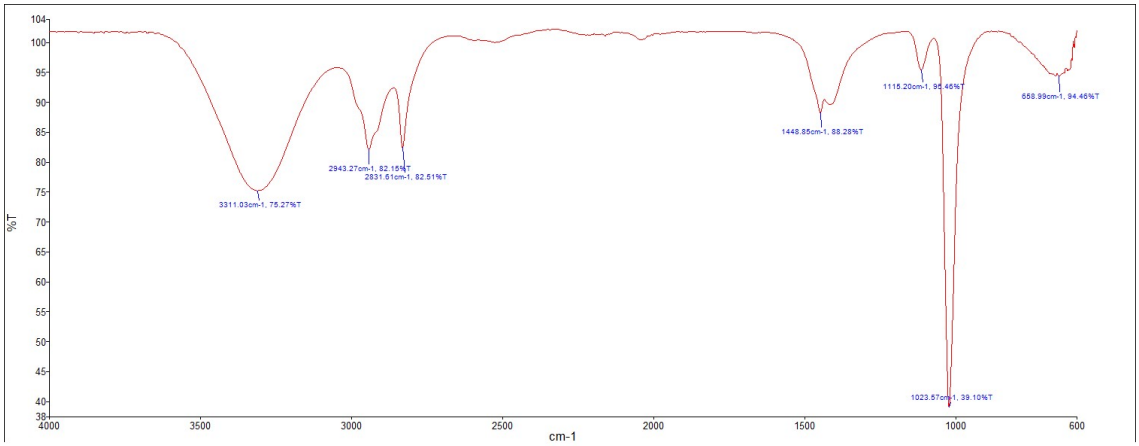


Figure 51: FTIR Spectrum of the Compound B of the Ethyl Acetate Extract (LEACB) of the *Persia americana* Leaf

LEACC.png

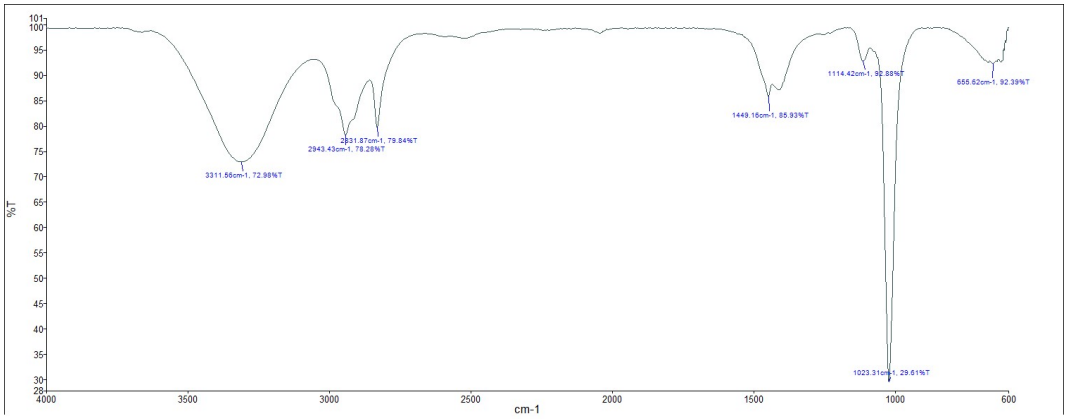


Figure 52: FTIR Spectrum of the Compound C of the Ethyl Acetate Extract (LEACC) of the *Persia americana* Leaf

LEACD.png

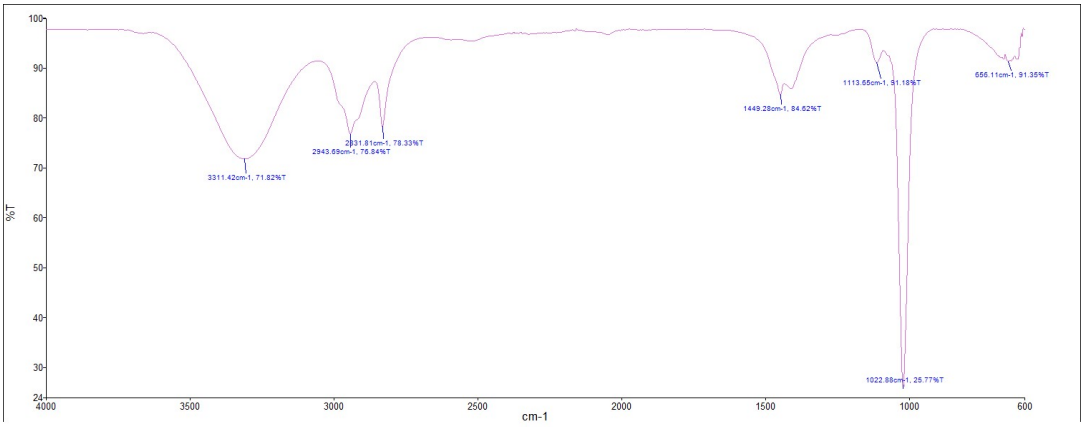


Figure 53: FTIR Spectrum of the Compound D of the Ethyl Acetate Extract (LEACD) of the *Persia americana* Leaf

RBCA.png

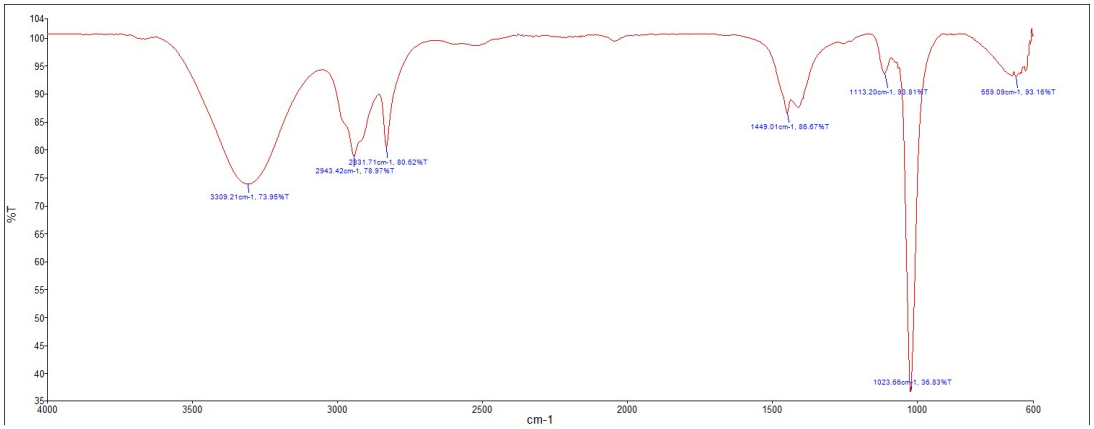


Figure 54: FTIR Spectrum of the Compound A of the Butanol Extract (RBCA) of the *Persia americana* Root

REACA.png

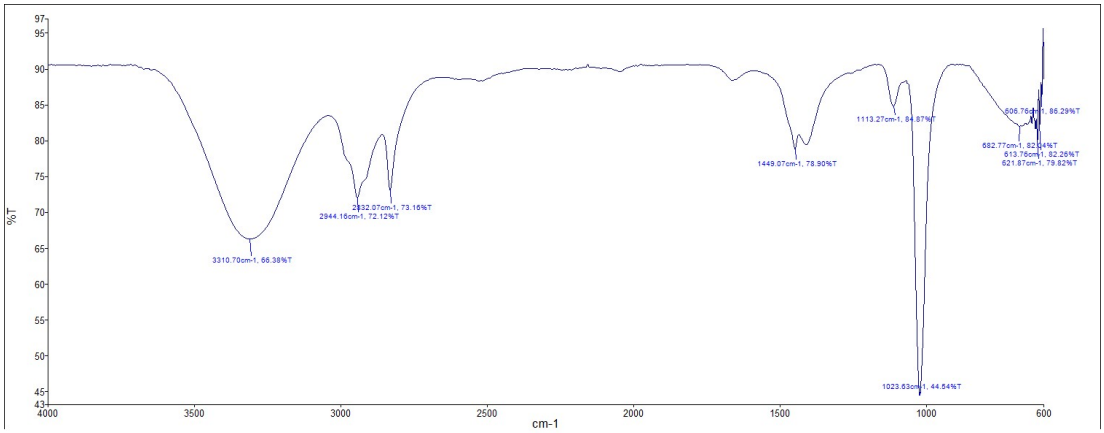


Figure 55: FTIR Spectrum of the Compound A of the Ethyl Acetate Extract (REACA) of the *Persia americana* Root

REACB.png

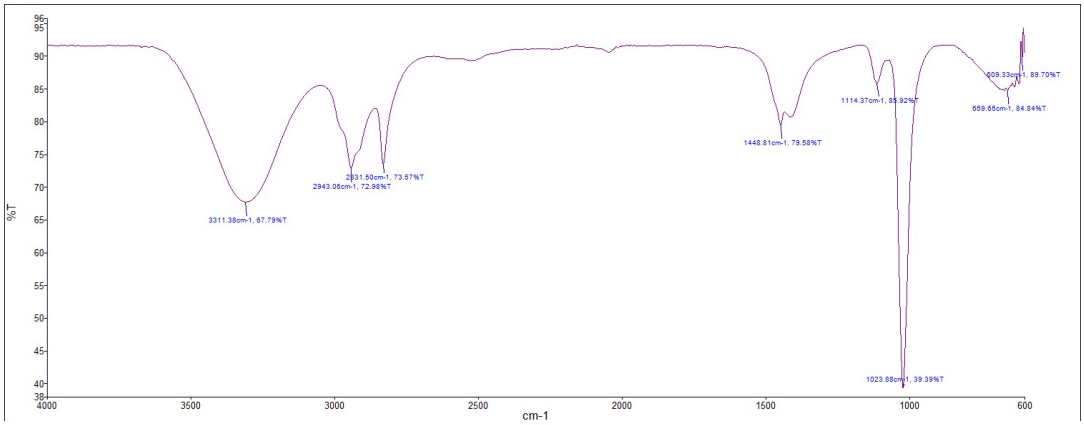


Figure 56: FTIR Spectrum of the Compound B of the Ethyl Acetate Extract (REACB) of the *Persia americana* Root

REACC.png

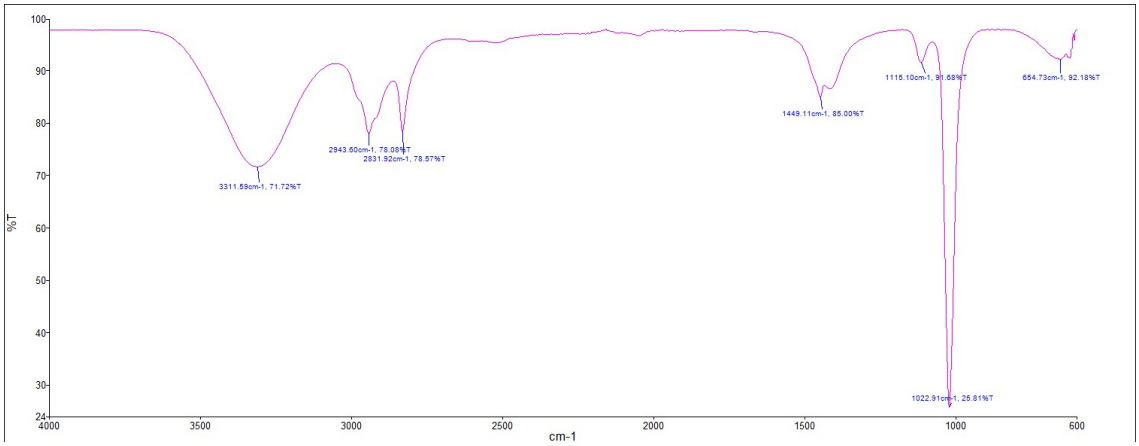


Figure 57: FTIR Spectrum of the Compound C of the Ethyl Acetate Extract (REACC) of the *Persia americana* Root

REACD.png

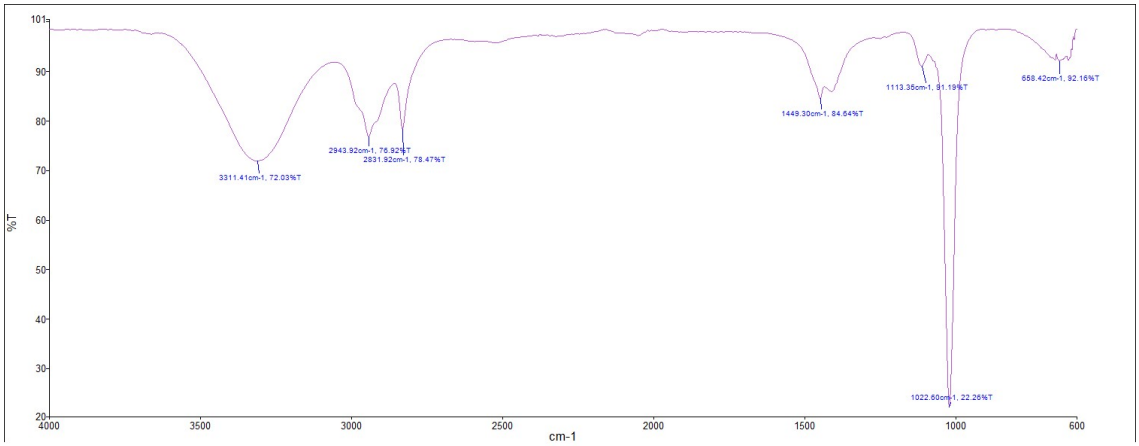


Figure 58: FTIR Spectrum of the Compound D of the Ethyl Acetate Extract (REACD) of the *Persia americana* Root

REACE.png

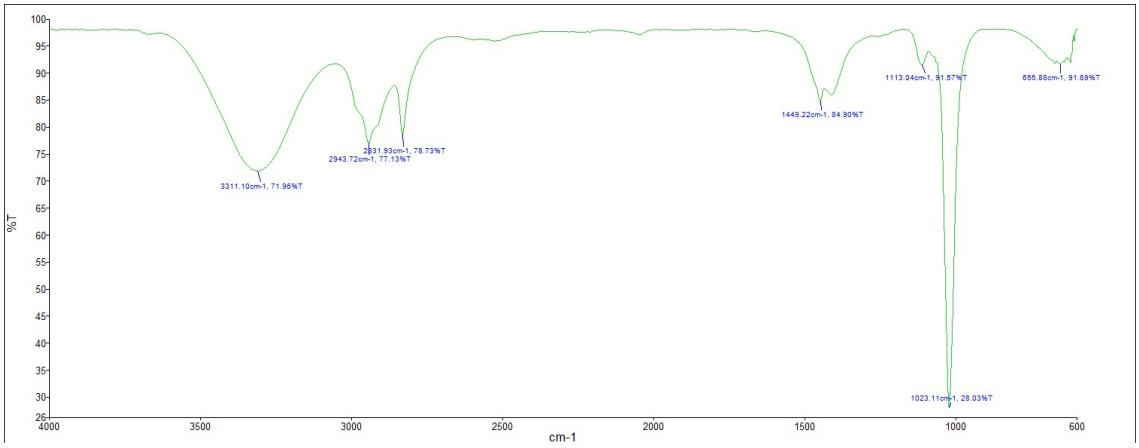


Figure 59: FTIR Spectrum of the Compound E of the Ethyl Acetate Extract (REACE) of the *Persia americana* Root

REACF.png

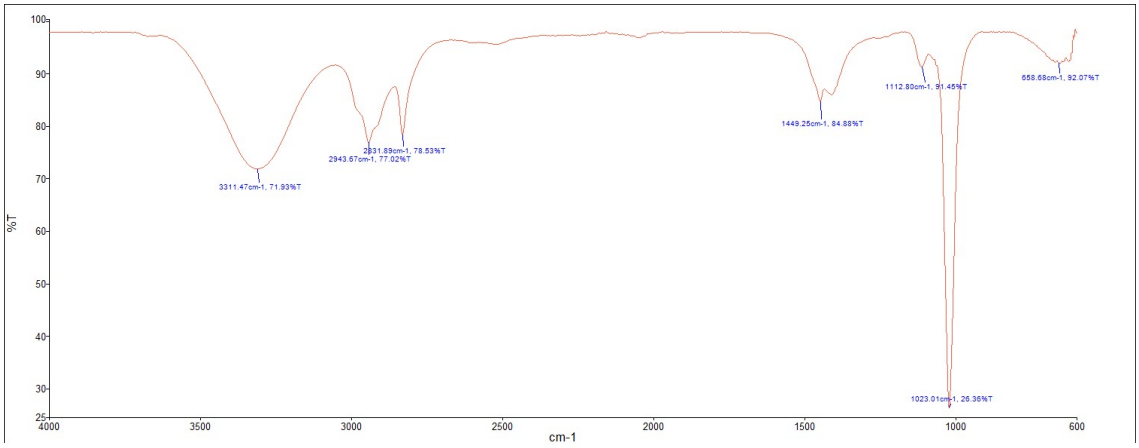


Figure 60: FTIR Spectrum of the Compound F of the Ethyl Acetate Extract (REACF) of the *Persia americana* Root

Figure 61: Elisa plate reader and printer

Figure 62: UV Spectophotometer

Figure 63: Camag UV lamp