

**EVALUATION OF ANTIOXIDANT PROPERTIES AND IN VITRO ACTIVITY OF A
POLYHERBAL INDIGENOUS ANTIHYPERTENSIVE TEA USING THESE ASSAY:
DPPH, AND FRAP.**

BY

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CERTIFICATION

We the undersigned hereby certify that this project was successfully carried out by ERAGBAI TOLULOPE JEMIMA (BMS2201813), in the Department of Medical Biochemistry, University of Benin, Benin City and thereby approve same as adequate in scope and quality for the award of Bachelor of Science Degree (B.Sc.) in Medical Biochemistry.

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DEDICATION

This research is dedicated to the Almighty God, who has blessed me with the strength, wisdom, and opportunity to complete this project, dedicate this work to Him, in humility and gratitude, acknowledging His Sovereignty and Provision in my life.

I also dedicate this project as a token of appreciation to Supervisor, Coordinators, lecturers and friends, and also to my mum, and to my sister and everyone in one way or another, who has contributed to my journey. Your support, encouragement, and guidance have been invaluable, and I am truly grateful for your presence in my life.

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ABSTRACT

Hypertension is a major public health challenge globally and has contributed significantly to cardiovascular morbidity and mortality. It is caused by oxidative stress, endothelial dysfunction, and lifestyle factors. Increasing interest in plant based alternatives has led to the use of polyherbal formulations, which provide synergistic antihypertensive and antioxidant effects. This study evaluated the antioxidant potential of six selected leaves which are, *Justicia carnea* (blood leaf), *Moringa oleifera* (miracle leaf), *Vernonia amygdalina* (bitter leaf), *Gongronema latifolium* (utazi leaf), *Hibiscus sabdariffa* (Zobo leaf) and *Telfairia occidentalis* (fluted pumpkin). Previous studies shows that these plants contain important bioactive constituents like flavonoids, phenols, and vitamins which promote vasodilation, reduce oxidative stress, and support cardiovascular health. A polyherbal tea was formulated from the six leaves to evaluate its antioxidant capacity. The leaves were collected, authenticated, washed, shade-dried, pulverized, and stored in an airtight container. 50g of each of the leaves was measured and mixed in 1L of hot distilled water in a ratio 5:1 to obtain aqueous extracts and then freeze-dried. The antioxidant activity of the extract was assessed using FRAP (Ferric Reducing Antioxidant Power) and DPPH (2,2-diphenyl-1-picrylhydrazyl) assays to measure to scavenge free radicals (DPPH assay) and to reduce ferric ions (FRAP assay). The result showed that the polyherbal extracts exhibited high antioxidant activity, with DPPH inhibition values of 71–85% and an IC₅₀ of 0.428 mg/mL, indicating good radical-scavenging ability. FRAP values (80–88%) confirmed high reducing power. Overall, this research confirms that the polyherbal tea possesses strong antioxidant compounds that can neutralize free radicals, support vascular health, and helps in the prevention and management of hypertension.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Hypertension remains a major public health challenge globally and contributes significantly to cardiovascular morbidity and mortality. In 2010, approximately 1.39 billion adults were affected with high blood pressure worldwide with 75% residing in the low and middle income countries (WHO, 2023). Hypertension is escalating and now affects about 30% of adults particularly in urban centers, largely due to lifestyles, high sodium dietary habits, and limited healthcare access.

In Nigeria, this rise significantly contributes to cardiovascular disease rates, such as stroke, heart failure, and kidney disease, amplifying the burden on the country's already strained healthcare system. A comprehensive analysis confirmed that several indigenous plants show strong antihypertensive effects in both humans and animal. (Oladele *et al.*, 2021). Medicinal plants have been used for centuries in traditional medicine systems worldwide for the prevention and treatment of various diseases. They serve as sources of bioactive compounds with therapeutic properties, including antihypertensive, antioxidant, anti-inflammatory, antidiabetic, and antimicrobial effects.

Herbal leaves play an important role in prevention of disease and health vitality because of their antioxidants and phytochemical properties. The following plants has been selected for this study, *Justicia carnea* (blood leaf), *Moringa oleifera* (miracle leaf), *Vernonia amygdalina* (bitter leaf), *Gongronema latifolium* (utazi leaf), *Hibiscus sabdariffa* (Zobo leaf) and *Telfairia occidentalis* (pumpkin leaf), and they are easily accessible and commonly consumed and among these,

Hibiscus sabdariffa has consistently demonstrated significant blood pressure lowering abilities (Oladele *et al.*, 2021).

The combination of these six herbs is expected to produce a synergistic effect, integrating their individual biochemical and nutritional benefits into a single formulation that supports the prevention and management of hypertension through natural and accessible means. The pharmacological effects of medicinal plants are attributed to their secondary metabolites, such as flavonoids which interact with biological targets to modulate physiological functions. The growing interest in herbal medicine underscores the need for further research and integration into modern healthcare systems.

1.2 AIM OF STUDY

The aim of this study was to evaluate the antioxidant scavenging activities of an antihypertensive herbal tea formulated from *Justicia carnea* (Hospital too far), *Moringa oleifera* (miracle tree), *Vernonia amygdalina* (bitter leaf), *Gongronema latifolium* (Utazi), *Hibiscus sabdariffa* (zobo leaf) and *Telfairia occidentalis* (fluted pumpkin), thereby establishing its possible role in the prevention and management of hypertension.

1.3 OBJECTIVES OF THE STUDY

The specific objective of this study were to;

1. Prepare a polyherbal indigenous tea using selected plant materials.
2. Evaluate the antioxidant scavenging properties of a formulated polyherbal tea.
3. Provide scientific justification for the traditional use of Nigerian herbal tea in the management of hypertension.

1.4 JUSTIFICATION OF STUDY

This study is justified for the need of safer, accessible and affordable remedies that can be assessed naturally and findings from this study will give a safe guide formulation of polyherbal tea and validate the extract's nutritional and health benefits as a conventional antihypertensive drugs that helps in managing hypertension and other related conditions.

CHAPTER TWO

LITERATURE REVIEW

2.1 OVERVIEW OF HYPERTENSION

2.1.1 Definition

Hypertension, commonly referred to as high blood pressure, is a chronic medical condition characterized by a persistent elevation in arterial blood pressure beyond normal physiological levels. The American Heart Association defines hypertension as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg, based on the average of two or more properly measured readings on separate occasions (Whelton *et al.*, 2020). It is one of the most significant risk factors for cardiovascular diseases such as stroke, heart failure, and chronic kidney disease (Mills *et al.*, 2020).

Globally, hypertension affects over 1.2 billion people, with the prevalence highest in low and middle income countries, where access to healthcare and preventive interventions are limited (World Health Organization, 2021). In Sub-Saharan Africa, the prevalence ranges between 30–40%, with many cases undiagnosed or poorly managed due to limited resources (Mills *et al.*, 2020). This condition has been described as a “silent killer” because it often remains asymptomatic in the early stages until complications arise.

2.1.2 Diagnosis of Hypertension

The diagnosis of hypertension relies on precise and consistent blood pressure assessment. This may be carried out manually using a sphygmomanometer or with automated blood pressure monitors for clinical and home use. Ambulatory blood pressure monitoring remains the gold

standard because it minimizes observer errors and provides repeated measurements throughout the day (Ott and Schmieder, 2022).

Blood pressure should be measured in both arms using properly sized upper-arm cuffs—finger cuffs are not recommended. The individual should be seated comfortably, relaxed, with feet flat on the floor and the arm supported at heart level (Ruopp and Cockrill, 2022). Ideally, readings are obtained with an empty bladder, taking at least two measurements five minutes apart, and repeating the process across two separate visits (Ruopp and Cockrill, 2022). In older individuals, blood pressure should also be checked for postural changes.

In addition to blood pressure readings, the diagnostic process includes laboratory investigations and electrocardiography, alongside evaluation of cardiovascular history, existing health conditions, and risk factors such as diabetes, stroke, heart failure, coronary artery disease, peripheral arterial disease, chronic kidney disease, and sleep disorders like sleep apnea (Ott and Schmieder, 2022). A comprehensive medical and medication history is essential for assessing overall risk and guiding appropriate management decisions.

Beyond blood pressure measurements, evaluation includes laboratory tests and electrocardiography, along with assessment of prior cardiovascular events, comorbidities, and risk factors such as stroke, coronary artery disease, heart failure, chronic kidney disease, peripheral artery disease, diabetes, and sleep apnea (Ott and Schmieder, 2022). A thorough medical and medication history guides risk stratification and informs treatment planning.

2.1.3 Blood Pressure Measurement

Blood pressure measurement remains the fundamental step in hypertension diagnosis and monitoring, serving both as a clinical indicator and a biochemical reflection of vascular function.

Accurate blood pressure (BP) assessment allows the detection of hemodynamic abnormalities that precede or accompany biochemical alterations such as oxidative stress, endothelial dysfunction, and vascular inflammation.

Traditional office based blood pressure measurement, while widely used, often fails to capture the full variability of blood pressure due to factors like white-coat or masked hypertension (Rhee et al., 2023). Recent evidence underscores the superiority of home and ambulatory BP monitoring, which provide a more reliable representation of 24-hour blood pressure patterns and are strongly associated with target organ damage and cardiovascular outcomes (Tomitani et al., 2023).

Modern hypertension evaluation now integrates biochemical and molecular markers with BP measurement to improve diagnostic accuracy. Advances in metabolomics studies have identified molecules such as acyl carnitines, amino acids, and lipid intermediates that correlate with both current and future hypertension risk, suggesting that metabolic dysregulation is a measurable biochemical signature of elevated blood pressure (Palmu et al., 2022). Moreover, plasma biomarkers like asymmetric dimethyl arginine (ADMA), endothelin-1, and C-reactive protein (CRP) provide insight into endothelial nitric oxide synthase (eNOS) activity and vascular inflammation, linking biochemical pathways to measurable hemodynamic changes (Liu et al., 2024). These markers not only assist in early detection but also serve as prognostic indicators for hypertension mediated organ damage.

Recent research also focuses on the integration of non invasive biochemical testing with continuous blood pressure monitoring, a strategy aimed at achieving “perfect 24 hour blood pressure management” (Tomitani et al, 2023). This combined approach enhances the predictive value of hypertension screening by accounting for both physiological and molecular

determinants of blood pressure regulation. While sphygmomanometer measurement remains essential, the incorporation of biochemical markers provides a deeper understanding of the molecular basis of hypertension, facilitating more individualized and preventive treatment strategies.

2.2 CLASSIFICATION OF HYPERTENSION

The classification of hypertension has evolved considerably over the years, driven by advances in understanding of the genetic mechanisms and clinical presentations. Hypertension can be broadly classified into primary (essential) hypertension and secondary hypertension.

Primary (essential) hypertension

This accounts for about 90–95% of diagnosed cases and has no clearly identifiable cause. It is thought to result from a complex combination of genetic, environmental cause, and lifestyle factors such as high salt intake, obesity, inactive lifestyle, stress and excess alcohol intake, (Carey *et al.*, 2021). It usually develops gradually over many years.

Secondary hypertension

The secondary hypertension accounts for 5–10% of cases that occur as a consequence of an underlying medical condition such as renal disease (chronic kidney disease), endocrine disorders (e.g., hyperaldosteronism, thyroid dysfunction, Cushing's syndrome), or as an adverse effect of certain medications like steroids, contraceptives (Carey *et al.*, 2021). This type, most times appears suddenly.

The European Society of Cardiology (ESC) and European Society of Hypertension (ESH) 2023 guidelines maintain the $\geq 140/90$ mmHg definition but emphasize early lifestyle and dietary

interventions at lower thresholds (Williams *et al.*, 2023). Biochemically, these classifications align with emerging insights into oxidative stress, renin-angiotensin-aldosterone system activation, and nitric oxide (NO) depletion as key mediators of endothelial dysfunction and vascular remodeling (Caminiti *et al.*, 2024). Thus, classification frameworks are not merely diagnostic but also mechanistic, reflecting the degree of redox imbalance and metabolic dysfunction associated with elevated BP. Functional foods rich in antioxidants such as polyphenols, flavonoids, and nitrate-containing vegetables play a complementary role by restoring endothelial function and attenuating oxidative stress (Thiruvengadam *et al.*, 2024). Recent international guidelines also categorize hypertension based on severity and clinical risk:

Elevated blood pressure: systolic 120–129 mmHg and diastolic <80 mmHg

Stage 1 hypertension: systolic 130–139 mmHg or diastolic 80–89 mmHg

Stage 2 hypertension: systolic ≥ 140 mmHg or diastolic ≥ 90 mmHg (Whelton *et al.*, 2020).

Understanding these classifications is essential in guiding therapeutic interventions and assessing the biochemical and physiological mechanisms underlying hypertension.

Table 2.1: Classification of Blood Pressure Levels in Adults (Whelton et al., 2018)

Category	systolic BP(mmHg)	Diastolic BP(mmHg)
Normal	<120	<80
Elevated (Prehypertension)	120-129	<80
Hypertension Stage 1	130-139	80-89
Hypertension Stage 2	≥ 140	≥ 90

2.2.1 Other Special Types of Hypertension

While primary and secondary hypertension constitute the majority of cases, several special forms of hypertension are clinically significant due to their distinct pathophysiology, prognosis, and management strategies. These types often present unique challenges in diagnosis and treatment, and their recognition is essential for optimizing patient outcomes.

Isolated Systolic Hypertension (ISH)

Isolated systolic hypertension is characterized by an elevated systolic blood pressure ≥ 140 mmHg with a normal diastolic pressure (< 90 mmHg). This condition is particularly prevalent in older adults and is primarily attributable to arterial stiffening and loss of vascular elasticity that occurs with aging (Whelton et al., 2020). ISH is strongly associated with increased risk of cardiovascular events, including stroke, heart failure, and myocardial infarction. The management of ISH often requires careful selection of antihypertensive agents that reduce systolic pressure without excessively lowering diastolic pressure, which could compromise coronary perfusion.

Resistant Hypertension

Resistant hypertension refers to blood pressure that remains above target despite the concurrent use of three or more antihypertensive agents at optimal doses, ideally including a diuretic. Patients with resistant hypertension often have underlying secondary causes, such as primary aldosteronism or renal artery stenosis, or contributing lifestyle factors like obesity, high salt intake, and non adherence to therapy (Carey et al., 2021). This form of hypertension carries a higher risk of cardiovascular morbidity and mortality, necessitating thorough evaluation to

identify reversible causes and the possible use of combination pharmacotherapy alongside lifestyle interventions.

Malignant or Accelerated Hypertension

Malignant hypertension, also referred to as accelerated hypertension, is a rapidly progressive and severe elevation of blood pressure, often defined as systolic BP ≥ 180 mmHg and/or diastolic BP ≥ 120 mmHg with evidence of end-organ damage, such as retinopathy, renal failure, or encephalopathy (Mills et al., 2020). This condition represents a medical emergency, requiring immediate antihypertensive treatment to prevent irreversible organ damage. Pathophysiologically, malignant hypertension involves widespread endothelial injury, activation of the renin-angiotensin-aldosterone system (RAAS), and microvascular ischemia, which collectively exacerbate vascular and tissue damage.

White Coat and Masked Hypertension

White coat hypertension is observed when patients exhibit elevated blood pressure readings in a clinical setting but normal readings outside the clinic, whereas masked hypertension presents with normal office readings but elevated blood pressure at home or during ambulatory monitoring (Tomitani et al., 2023). These phenomena underscore the importance of accurate and repeated blood pressure assessment, as misclassification may lead to inappropriate treatment or failure to intervene in high-risk individuals.

Pregnancy Related Hypertension

Pregnancy-induced hypertension (PIH) or gestational hypertension occurs in pregnant women after 20 weeks of gestation, with a blood pressure $\geq 140/90$ mmHg. When accompanied by proteinuria and organ dysfunction, it is classified as preeclampsia, a condition that increases

maternal and fetal morbidity and mortality (National Institute for Health and Care Excellence, 2022). Management requires careful monitoring and timely intervention to prevent severe complications such as eclampsia or preterm birth.

Special types of hypertension are clinically distinct entities that require tailored diagnostic and management approaches. They highlight the heterogeneity of hypertensive disorders and the importance of considering age, comorbidities, rapidity of onset, and environmental factors in treatment planning. Understanding these variations is critical for reducing cardiovascular risk, preventing target organ damage, and improving patient outcomes.

2.3 CLINICAL EVALUATION OF HYPERTENSION

2.3.1 Conventional Pharmacological Drugs and Therapy

The clinical management of hypertension typically begins with pharmacological therapy. The pharmacological management of hypertension is central to its clinical control and prevention of cardiovascular complications. The current guidelines from the European Society of Hypertension (ESH) recommend initiating therapy with one of four major classes of antihypertensive drugs: Angiotensin Converting Enzyme Inhibitors (ACEIs), Angiotensin Receptor Blockers (ARBs), Calcium Channel Blockers (CCBs), and Thiazide or Thiazide-like diuretics, either as monotherapy or combination therapy (Mancia et al., 2023).

1. Angiotensin-converting enzyme (ACE) inhibitors: (e.g., enalapril), which block the conversion of angiotensin I to angiotensin II, thereby reducing vasoconstriction and aldosterone secretion. ACE inhibitors, act by blocking the conversion of angiotensin I to angiotensin II, resulting in vasodilation and reduced aldosterone-mediated sodium

retention, which contributes to long term blood pressure control (Goyal and Johnson, 2023).

2. Angiotensin II receptor blockers (ARBs): (e.g., losartan), which inhibit angiotensin II receptors on vascular smooth muscle. Beta-blockers (e.g., atenolol), which lower cardiac output and inhibit renin release (Carey et al., 2021).
3. Calcium channel blockers (CCBs): (e.g., amlodipine), which prevent calcium entry into vascular smooth muscle, leading to vasodilation. CCBs are particularly useful for elderly patients and those with isolated systolic hypertension because of their ability to promote arterial compliance and prevent vasospasm (Jones, 2024).
4. Diuretics: (e.g., thiazides), which reduce blood pressure by promoting sodium and water excretion, thus lowering plasma volume. Among these, thiazide diuretics remain a cornerstone for uncomplicated hypertension due to their efficacy in reducing stroke and coronary events (Reinhart, 2023).

These medications are often prescribed alone or in combination, depending on disease severity, comorbidities, and patient response. In clinical evaluation, antihypertensive treatment choice is guided by patient comorbidities, race, and biochemical profile. The use of ACEIs and ARBs is often preferred for patients with diabetes or chronic kidney disease, while beta-blockers are reserved for those with concomitant ischemic heart disease or arrhythmia (Mancia et al., 2023). Combination therapies typically ACEI/CCB or ARB/thiazide pairs, improve adherence and therapeutic outcomes. Despite pharmacological advances, optimal blood pressure control rates remain suboptimal globally, highlighting the need to integrate functional-food-based or antioxidant dietary interventions as adjuncts to conventional treatment (Reinhart, 2023).

2.3.2 Biochemical Mechanisms

The effectiveness of conventional antihypertensive drugs is linked to their ability to modify biochemical pathways involved in blood pressure regulation. The biochemical mechanisms underlying the action of conventional antihypertensive drugs involve modulation of neurohormonal and oxidative pathways central to vascular homeostasis. ACE inhibitors suppress the Renin Angiotensin Aldosterone system (RAAS). ACE inhibitors and ARBs interrupt this pathway, lowering Angiotensin II levels and preventing sodium retention and vasoconstriction (Zheng, 2022). By inhibiting the Angiotensin Converting enzyme, reducing the formation of angiotensin II, a potent vasoconstrictor and preventing bradykinin degradation, thereby promoting nitric oxide (NO) mediated vasodilation (Zheng, 2022). In parallel, ARBs block angiotensin II binding to the AT1 receptor, attenuating oxidative stress and vascular inflammation through suppression of NADPH oxidase driven reactive oxygen species (ROS) generation (Montezano and Touyz, 2021).

Diuretics decrease extracellular fluid volume and cardiac preload, and cardiac output, ultimately lowering blood pressure. While long term effects involve alterations in vascular reactivity and endothelial signalling (Morales-Olivas, 2024). The interaction between oxidative stress and hypertension is pivotal to understanding drug mechanisms. Calcium Signaling, CCBs block calcium influx, which reduces vascular smooth muscle contraction and systemic vascular resistance (Opie and Messerli, 2020). Sympathetic Nervous System (SNS), the Beta blockers suppress adrenergic activity, decreasing heart rate, myocardial contractility, and renin release (Carey et al., 2021).

Excess ROS, largely produced via mitochondrial leakage and NOX enzyme activation, reduces NO bioavailability and leads to endothelial dysfunction. Pharmacological and nutritional

antioxidants both counter this by re-establishing redox equilibrium (Caminiti et al., 2024). Collectively, antihypertensive drugs exert pleiotropic effects beyond pressure reduction by improving endothelial function, reducing oxidative damage, and mitigating vascular remodelling. These molecular mechanisms support the rationale for combining pharmacotherapy with natural antioxidant interventions to achieve synergistic cardiovascular protection.

2.3.3 Limitations of Conventional Drugs

Despite the therapeutic success of antihypertensive drugs, several limitations hinder their efficacy and long term outcomes. Poor medication adherence remains one of the foremost challenges, particularly among older adults with multimorbidity or complex drug regimens (Kim et al., 2024). Non-adherence is often driven by adverse side effects, high bill burden, and inadequate patient education, which collectively reduce treatment effectiveness and increase cardiovascular risk (Lee et al., 2024). The side effects may varies, patients may experience dizziness, electrolyte imbalances, persistent cough (ACE inhibitors), or fatigue (beta-blockers).

Moreover long term daily medication often results in poor compliance, especially in low resource settings. A subset of patients develops resistant hypertension defined as blood pressure that remains uncontrolled despite the use of three or more antihypertensive agents at optimal doses which complicates management and necessitates careful evaluation for secondary causes (European Society of Cardiology, 2024).

In developing countries, access to medications is often limited, leading to high rates of uncontrolled hypertension (Mbayo et al., 2020). Genetic, dietary, and environmental factors also influence drug response, leaving some patients resistant to treatment (Baptista et al., 2021). Diagnostic limitations also contribute to suboptimal hypertension control. Office blood pressure

measurement alone may yield inaccurate readings due to white-coat or masked hypertension effects, whereas ambulatory or home blood pressure monitoring offers better diagnostic precision and risk stratification (Lee et al., 2024). However, these techniques are underutilized in low resource settings because of equipment costs and limited awareness.

Finally, pharmacotherapy does not directly address the oxidative and endothelial dysfunction that underlie hypertension pathogenesis. This limitation reinforces the growing advocacy for adjunctive nutritional strategies particularly antioxidant-rich functional foods to complement drug therapy and improve biochemical outcomes (Caminiti et al., 2024). These challenges underscore the need for complementary and alternative approaches, including nutritional and herbal interventions, which may provide additional benefits in hypertension management.

2.3.4 Non-Pharmacologic Management of Hypertension

Lifestyle modification is a fundamental component of hypertension management and is recommended alongside medication for all patients. Essential interventions include weight loss, limiting dietary sodium, engaging in regular aerobic activity, quitting smoking, and reducing alcohol consumption (Tjahjono, 2024). These behavioural changes have consistently been shown to lower systolic blood pressure in individuals with both hypertension and pre-hypertension.

Table 2.2: Management of Hypertension (Verma et al., 2021)

Pharmacological Management	Non-pharmacological Management
ACE inhibitors	Lifestyle modifications
ARBs; Tehiartan	Physical activity
Beta blocker	Weight management

Calcium channel blockers	Limited alcohol consumption
Diuretics	Reduced sodium Intake
Centrally acting Adrenergic agonists	DASH diet
Alpha blockers	
Direct vasodilators	

2.4 A POLYHERBAL ANTIHYPERTENSIVE TEA: A NUTRITIONAL INTERVENTION

Polyherbal formulations is the combination of two or more medicinal plants to enhance therapeutic efficacy and minimize adverse effects through synergistic interactions. This concept, which originates from traditional medicine systems such as African, Ayurveda, and Chinese ethno medicine, is based on the fact and conclusion that the collective activity of several bioactive compounds of the herbal medicine provides more balanced and effective healing effects than individual or single plant remedies (Adeyemi and Fagbohun, 2020; Singh *et al.*, 2021). In this regard, polyherbal teas have become increasingly common due to their accessibility, safety, and effectiveness in managing life related ailments or diseases such as diabetics and hypertension.

Hypertension, also known as high blood pressure is a chronic medical condition associated with an elevation of arterial blood pressure. It is a major risk factor for stroke, cardiovascular diseases, and renal complications. Although there are many synthetic antihypertensive drugs but due to their high cost and long term use and also the side effects they are limited in consumption. As a result, there has been a growing interest in plant based alternatives that regulate blood pressure

through multiple mechanisms (Mohammed *et al.*, 2022) . Polyherbal teas for hypertension are formulated with herbs that possess complementary actions, such as improving kidney function, promoting vasodilation, reducing lipid accumulation, and combating oxidative stress which together contribute to cardiovascular protection and blood pressure regulation (Yakubu, *et al.*, 2020).

From a biochemical perspective, polyherbal teas are rich in diverse phytochemicals, including flavonoids, saponins, polyphenols, alkaloids, and terpenoids. These bioactive compounds interact with physiological systems to enhance vascular elasticity, scavenge free radicals, and modulate enzymes involved in blood pressure control (Chukwuma *et al.*, 2021). In particular, polyphenols and flavonoids function as natural antioxidants that neutralize reactive oxygen species (ROS), thereby reducing oxidative damage that is linked to cardiovascular disease such as hypertension and others (Hussain *et al.*, 2020).

Furthermore, polyherbal teas also contribute nutritionally through the provision of vital vitamins, minerals, and essential micronutrients such as calcium, potassium, magnesium, phosphorus, iron, and zinc. These nutrients play vital roles in cardiac and vascular health, for example, calcium is responsible for regulating blood pressure, heart muscle contraction and vascular tone, thereby reducing arterial resistance and promoting vasodilation. Magnesium aids smooth muscle relaxation, while potassium supports electrolyte balance and reduces vascular tension (Mbatha and Ogunlakin, 2023). According to Okolie *et al.*, (2022), Zinc is also an essential trace elements that supports vascular integrity enzyme function, and antioxidant defense, beyond their pharmacological properties, polyherbal teas serve as nutritional interventions that promote overall wellbeing while addressing specific health challenges.

Overall, polyherbal teas represent an integrative approach to health management. Rather than targeting a single symptom, they help correct the underlying physiological imbalances that lead to conditions like hypertension. Their combined nutritional, therapeutic and preventive effects underscore their growing significance in both traditional and modern healthcare research (Yakubu *et al.*, 2020).

2.4.1 Concept of Polyherbal Teas

Polyherbal teas are beverages or liquid substances that are made from different mixture of medicinal plants and are consumable for both their nutritional and therapeutic benefits. Unlike individual or single herbs preparations, polyherbal teas are formulated based on the principle of synergy, whereby the combining of the activity of several plants enhances effectiveness and reducing side effects (Singh *et al.*, 2021). That means that when two or more plants are combined together, they tend to produce more results that is more efficient and stronger with little or no effect than when it is used individually. Traditionally, this synergistic effect or combination of herbal medicine has been in existence whereby healers or herbalists combine plants with complementary actions such as diuretic, antioxidant, and vasodilatory properties to promote cardiovascular health. They believed single herbs cannot provide all the healing properties needed to treat a complex ailments or health conditions like diabetes or hypertension. So the concluded that herbal medicine formulated from the combination of two or more leaves have more complementary actions like antioxidant and vasodilatory actions. These formulations are natural sources as they are rich in bioactive compounds, particularly polyphenols, and flavonoids which both contribute to their antihypertensive potential.

From a biochemical standpoint, polyherbal teas serve as a rich sources of diverse phytochemicals such as flavonoids, alkaloids, polyphenols, terpenoids, and saponins. These bioactive compounds

collectively interact with the body's physiological systems involved in the regulation of blood pressure, improve vascular elasticity, and reduction of oxidative stress (Chukwuma *et al.*, 2021). In particular, the flavonoids and polyphenols act as natural antioxidants that can neutralize reactive oxygen species (ROS), which are known contributors to cardiovascular disorders by reducing the oxidative damage associated to it (Hussain *et al.*, 2020) .

Another importance of these polyherbal teas is their safety and accessibility. Because they are majorly derived from edible or commonly used medicinal plants, they are often considered safer and more affordable thereby access able than conventional or synthetic drugs, especially in low-resource countries (Mohammed *et al.*, 2022). Moreover, these teas also contribute nutritionally, through the provision of important minerals, vitamins and other essential micronutrients thereby making them more suitable for long term consumption or use as functional beverages that helps manage and treat specific ailments in addition to also promoting the overall wellbeing (Mbatha and Ogunlakin, 2023).

In essence, polyherbal teas represent a comprehensive approach to health management. Instead of targeting a single symptom, they address and correct the underlying physiological imbalances that lead to conditions such as hypertension. The herbal teas ability to combine nutritional value with therapeutic benefits and preventive care lead to their growing recognition and importance in both traditional and modern healthcare research (Yakubu *et al.*, 2020).

2.4.2 Antihypertensive Polyherbal Teas in Research

Studies have shown the efficacy of the properties of polyherbal teas and has support the use of polyherbal teas in the management of hypertension. For example, combining the mixture of Hibiscus sabdariffa, Vernonia amygdalina and Moringa oleifera have been reported to reduce

blood pressure through vasodilation by improving vascular relaxation, and promoting production of urine and antioxidant activity (Adeoye and Akinloye, 2020). In both clinical and experimental studies, teas rich in phenolic compounds have been proven to have the ability to reduce diastolic and systolic blood pressure, enhance lipid metabolism and improve vascular function (Chukwuma *et al.*, 2021). These findings highlights the potential of polyherbal teas, when formulated appropriately, may serve as effective complementary therapies for hypertension.

2.4.3 Traditional Uses of Polyherbal Teas

In African and Asian societies, herbal teas have long been used in the prevention and management of chronic diseases. These herbal mixtures are usually prepared from a combination of leaves, barks, roots or seeds of medicinal plants and are healthy for consumption to promote the general wellbeing and prevention and treatment of common ailments (Adeyemi and Fagbohun, 2020). In many indigenous cultures, polyherbal formulations are believed to provide synergistic healing effects, when there is a combination of multiple plants enhances potency and reduces potential side effects compared to single herb remedies.

In Nigeria, combinations of leafy vegetables and medicinal plants are commonly prepared as teas or decoctions to manage conditions such as diabetes, hypertension, malaria, fatigue, inflammation, digestive disorders and other related conditions (Adebisi and Ibrahim, 2022). They are also act as immune boosting and detoxifying substances, often consumed to cleanse the blood, support the kidney and liver function and also improve blood circulation. Traditionally, herbalists emphasize that blends of plants provide therapeutic advantages by offering a wider spectrum of bioactive compounds. Such uses highlight the ethno medical importance of polyherbal teas in supporting cardiovascular health and general well-being. The continued use of these teas reflects

the deep integration of herbal therapy into cultural healthcare practices, where they serve both nutritional and therapeutic purposes.

2.4.4 Nutritional Value of Polyherbal Teas

Polyherbal teas are not only consumed for their therapeutic purposes but also contribute to daily nutrition. They supply essential vitamins such as A, C and E, minerals including magnesium, zinc, potassium and calcium, as well as antioxidants like phenolic acids and flavonoids (Tońska *et al.*, 2021). These nutrients play a dual role, firstly they support cardiovascular health function and then reducing oxidative stress, which is an important factor in the development of hypertension (Iqbal *et al.*, 2023). Regular intake of these polyherbal teas enhances the dietary antioxidant intake while contributing to long-term prevention of cardiovascular diseases.

2.5 NUTRITIONAL AND BIOCHEMICAL PROPERTIES OF RAW MATERIALS USED IN THIS STUDY

The raw materials selected for the formulation of the polyherbal antihypertensive tea possess distinct nutritional and biochemical attributes that contribute to its overall therapeutic efficacy. Medicinal plants are rich sources of bioactive compounds such as flavonoids, alkaloids, phenolic acids, tannins, terpenoids, and saponins, which play crucial roles in antioxidant defense, lipid metabolism, and blood pressure regulation (Osagiede *et al.*, 2024).

The leaves selected for this study of the formation of polyherbal antihypertensive tea that is, *Justicia carnea*, *Moringa oleifera*, *Vernonia amygdalina*, *Gongronema latifolium*, *Hibiscus sabdariffa*, and *Telfairia occidentalis*, were chosen because of their scientific proven medicinal potential and also their rich nutritional value. Each of the plants has their unique micronutrients and bioactive components that they contribute that work together to promote cardiovascular

health and the overall wellbeing of an individual. From a nutritional perspective, these plants provide essential vitamins (A, C, E, and B-complex) and minerals (Fe, Zn, Ca, Mg, K, and P) necessary for maintaining normal physiological functions. Minerals like magnesium and potassium aid in vascular relaxation and electrolyte balance, while iron and zinc support oxygen transport and enzymatic reactions involved in cardiovascular protection (Eguaoje *et al.*, 2024).

Biochemically, the phytochemicals present in the raw materials exhibit antioxidant, anti-inflammatory, diuretic, and vasodilatory properties. For instance, flavonoids and polyphenols scavenge free radicals, thereby preventing oxidative stress, a major contributor to endothelial dysfunction and hypertension (Anameze *et al.*, 2023). Saponins and tannins may further contribute to lipid-lowering and blood vessel-stabilizing effects, enhancing cardiovascular resilience (Eguaoje *et al.*, 2024).

Collectively, the nutritional and biochemical constituents of the selected herbs function synergistically to restore vascular balance, improve circulatory efficiency, and promote overall wellbeing. This combination of nutritional and pharmacological benefits underscores the significance of using multiple plant species in polyherbal formulations for managing hypertension and related disorders.

2.5.1 *Justicia carnea* (blood leaf)

Justicia carnea is an edible leafy plant commonly known as “blood leaves” or “hospital too far”. It is traditionally recognized for its appreciable nutrient content and dietary relevance and also used in West Africa as a blood tonic and for the treatment of anaemia. Recent proximate analyses indicate that the leaves is rich in carbohydrates (approximately 60%), providing an important source of energy for human nutrition. In addition, *Justicia carnea* contains moderate

protein levels (about 8–9%) and dietary fibre (~9%), which support digestive health and metabolic regulation. The fat content is relatively low, averaging around 1.5%, making the leaves suitable for low-fat diets (Chimezie et al., 2024). Moreover, the plant is a rich source of vitamins, particularly vitamin C (232.32 mg/100 g) and vitamin A (22.16 mg/kg), both of which play essential roles in immune defense, collagen synthesis, and vision maintenance (Ayodele *et al.*, 2024).

The plant is rich in bioactive compounds such as flavonoids and other antioxidant (Akinmoladun *et al.*, 2020). Phytochemical studies have also revealed appreciable amounts of iron and other minerals that support hematopoiesis, alongside antioxidants that help protect cells against oxidative stress (Andrew *et al.*, 2024). The mineral composition of *Justicia carnea* leaves is also nutritionally significant. Studies have shown that the plant contains considerable amounts of iron (8.61 mg/kg) and potassium (5.29 mg/kg), alongside other minerals such as calcium, magnesium, and phosphorus, which contribute to hemoglobin synthesis and electrolyte balance (Chimezie *et al.*, 2024). When compared with other leafy vegetables, *Justicia carnea* exhibits comparable or even higher micronutrient concentrations, underscoring its potential as a valuable dietary component in combating micronutrient deficiencies (Ayodele *et al.*, 2024). These properties make *Justicia carnea* a medicinally and nutritionally valuable component of polyherbal formulations aimed at improving overall nutritional quality.

2.5.2 *Moringa oleifera* (Moringa leaf)

Moringa oleifera is commonly known as Miracle tree or Moringa leaves. It is also known for its nutritional richness. Its leaves contain high levels of vitamins A, C and E, minerals such as calcium, potassium and magnesium, as well as essential amino acids (MDPI, 2023). Biochemically, it is an abundant source of flavonoids, phenolic acids, glycosylates and

isothiocyanates, which exhibit antioxidant, anti-inflammatory and cardio protective effects (Nnadi and Igbokwe, 2022). Studies have shown that *Moringa oleifera* extracts can reduce blood pressure by improving vascular elasticity and endothelial function and reducing oxidative stress (Nnadi and Igbokwe, 2022).

2.5.3 *Vernonia amygdalina* (Bitter leaf)

Vernonia amygdalina (commonly called bitter leaf) is widely used in African traditional diets and medicine that has both nutritional value and therapeutic effects. Nutritionally, recent studies has showed that it provides fibre about 15%, proteins about 21%, and micronutrients such as zinc and iron (Adewuyi *et al.*, 2025). Its phytochemicals include flavonoids, sesquiterpene, polyphenols, sesquiterpene lactones which have antioxidant, detoxifying and anti-inflammatory properties (Adewuyi *et al.*, 2025). The bitter taste of the *Vernonia amygdalina* stimulate bile secretion and also assist in maintaining the normal cholesterol levels. The use of experimental animals, has showed evidence of blood lowering effects as it reduces both the systolic and diastolic blood pressure in hypertensive Wistar rats suggests through vasodilation (Afolabi *et al.*, 2023). Therefore *Vernonia amygdalina* has antihypertensive potential by modulating vascular resistance and reducing oxidative stress.

2.5.4 *Gongronema latifolium* (Utazi leaf)

Gongronema latifolium is locally called “Utazi” is a leafy vegetable used both as a spice and as medicine in Nigeria. It is a good source of vitamins, minerals and phytochemicals such as alkaloids, saponins and flavonoids (Nwuneli *et al.*, 2022). The plant has been shown to exert antioxidant, antidiabetic and antihypertensive activities. Its flavonoid and polyphenolic contents from the regular consumption of the leave has showed to play a role in scavenging free radicals

and improving vascular health (Analike and Ahaneku, 2022). They contribute to blood pressure control by regulating blood glucose and enhancing vasodilation.

2.5.5 *Hibiscus sabdariffa* (Zobo leaf)

Hibiscus sabdariffa is locally called Zobo leaves. Its calyces are widely consumed as herbal teas. They are rich in anthocyanins, flavonoids, vitamin C and organic acids, which contribute to their strong antioxidant capacity which exhibit strong lipid lowering and antihypertensive effects (Sani *et al.*, 2020). Clinical studies have reported that hibiscus leaf tea can significantly lower systolic and diastolic blood pressure, attributed mainly to its vasodilatory and diuretic effects as well as supporting kidney function by the inhibition of angiotensin-converting enzyme (ACE) (Negida *et al.*, 2021). These properties make it an important ingredient in antihypertensive herbal preparations.

2.5.6 *Telfairia occidentalis* (fluted pumpkin leaf)

Telfairia occidentalis is commonly known as pumpkin leaves and locally called “Ugwu” leaves. It is a common vegetable in West Africa, often used in to make soups and medicinal herbs. Nutritionally, its leaves provide proteins, essential amino acids, vitamins A, C and E, iron, and other trace elements like zinc, iron (Nwuneli and Ishiwu, 2022). The plant also contains polyphenols, alkaloids and flavonoids, which have been linked to antioxidant, antidiabetic and antihypertensive properties (Ilechukwu *et al.*, 2023). Its nutritional and phytochemical profile supports its inclusion in polyherbal formulations aimed at cardiovascular health. They support red blood cell formation, heart function and immunity.

2.6 ROLE OF DIET AND ANTIOXIDANTS IN HYPERTENSION MANAGEMENT

2.6.1 Natural Antioxidants

Natural antioxidants are compounds derived mainly from plants that protect the body against oxidative damage caused by free radicals. They reduce or neutralizes free radicals that have gained substantial attention for their potential role in modulating the early pathophysiology of hypertension. Oxidative stress and endothelial dysfunction are key early events in the progression toward sustained elevated blood pressure, and that natural antioxidants may support vascular health by preserving endothelial integrity (Caminiti *et al.* 2024).

Natural Antioxidants include dietary derived antioxidants such as vitamins (A, C, and E), minerals such as selenium and zinc, as well as polyphenols and flavonoids present in fruits, vegetables, and medicinal herbs (Nutrition Journal, 2024). These substances also include endogenous enzymes like superoxide dismutase (SOD), glutathione peroxidase, catalase. The review emphasizes that by reducing reactive oxygen species (ROS) burden, natural antioxidants improve nitric oxide (NO) bioavailability, mitigate lipid peroxidation and inhibit vascular-remodelling processes (Caminiti *et al.*, 2024). By intervening at this early stage the conversion of oxidative stress into endothelial dysfunction, antioxidant-rich dietary patterns may contribute to preventing the development of hypertension rather than merely treating established high blood pressure.

In this context, natural antioxidants act not only as free-radical scavengers but also as modulators of vascular signaling pathways and structural vascular changes. These compounds are particularly important in cardiovascular health, since they help maintain vascular integrity, reduce lipid peroxidation, and prevent oxidative stress, a key contributor to hypertension. Diets rich in natural

antioxidants have been associated with lower blood pressure and improved endothelial function (National Research Centre, 2025).

2.6.2 Biochemical Mechanisms linking Oxidative Stress to Hypertension

The biochemical mechanisms by which oxidative stress drives endothelial dysfunction and hypertension and how natural antioxidants interrupt these processes. Oxidative stress plays a major role in the pathogenesis of hypertension through its impact on vascular cells and function. They are two types of cells inside the blood vessels, the endothelial cells and vascular smooth muscle cells (VSMCs) generate reactive oxygen species (ROS) primarily via mitochondrial respiratory chain leak and nicotinamide adenine-dinucleotide phosphate oxidase (NOX) enzymes (NOX1, NOX2, NOX4, NOX5) especially when the blood vessels are under stress or pressure . The review notes that in conditions of elevated intravascular pressure or stretch, NOX enzymes are activated, thereby producing superoxide anion which is a strong oxidant and can accelerate inflammation, lipid peroxidation, vascular contractility and remodeling (Caminiti *et al.*, 2024). Another major driver is the renin–angiotensin system (RAS). The angiotensin II further amplifies ROS via the binding of angiotensin II to AT1 receptors, leading to the stimulation of excess NOX enzymes, increasing the ROS levels and raising blood pressure (Caminiti *et al.*, 2024).

The antihypertensive role of antioxidants in hypertension arises from their ability to counteract these oxidative processes by neutralizing or reducing reactive oxygen species (ROS) before they cause damage. ROS accumulation, largely mediated through their ability of restoring redox balance within vascular tissues. Excess ROS production in vascular tissues can lead to impaired nitric oxide (NO) availability, leading to endothelial dysfunction, and increased vascular and peripheral resistance, all of which contribute vascular dysfunction in hypertension (Rodrigo *et al.*, 2021). Antioxidants help restore nitric oxide bioavailability which is a molecule that is essential

for endothelial relaxation, promote vasodilation, and protect vascular smooth muscle from oxidative injury (overall, they help the blood vessels to relax). Polyphenolic antioxidants in particular modulate intracellular signaling pathways and inhibit angiotensin-converting enzyme (ACE), which helps lower blood pressure (Schini-Kerth *et al.*, 2020). Natural antioxidants intervene by restoring redox balance: scavenging free radicals, chelating metal ions that catalyze radical formation, inhibiting NOX activation, preserving eNOS coupling and improving NO mediated vasodilation (Caminiti *et al.*, 2024). For example, dietary antioxidants may help to stabilize the BH₄ co-factor of eNOS (the enzyme that produces nitric oxide), prevent superoxide mediated NO inactivation, and thereby maintain the endothelium's ability to regulate vascular tone. Antioxidant mechanisms align with upstream vascular signalling events, offering a biologically plausible route to influence early hypertension development.

Overall, the biochemical interplay between oxidative stress and vascular function highlights how natural antioxidants, particularly those present in polyherbal teas, can disrupt this harmful cycle. By scavenging ROS and stabilizing endothelial signaling, these phytochemicals help protect vascular integrity and regulate blood pressure, reinforcing their therapeutic relevance in hypertension management.

2.6.3 Functional Herbal Teas in Hypertension

Herbal teas are increasingly recognized as functional foods or beverages that contribute to both the management and prevention of hypertension. These teas have bioactive compounds that exert health-promoting effects beyond basic nutrition. Teas prepared from the synergistic combination of *Hibiscus sabdariffa*, *Moringa oleifera* and other medicinal plants are not only considered to be available for consumption but also has proven to have antihypertensive properties in both clinical and experimental studies because of their rich polyphenolic and flavonoid contents (Anyanwu *et*

al., 2023). These compounds help to reduce oxidative stress, enhance vasodilation, and support renal excretion of sodium. Regular consumption of such teas has been associated with improvements in blood pressure, lipid profiles, and overall cardiovascular health (Olatunji *et al.*, 2020).

Clinical and observational studies suggest that herbal teas enriched with specific bioactive phytochemicals such as peptides, flavonoids, polyphenols, nitrates (from vegetables such as *Justicia carnea*), and mineral-rich constituents can yield measurable reductions in systolic and diastolic blood pressure (Usman *et al.*, 2022). Polyherbal tea containing ingredients like *Moringa oleifera*, *Hibiscus sabdariffa* (Zobo), *Vernonia amygdalina* (bitter leaf), *Gongronema latifolium* (utazi), and pumpkin leaves deliver a diverse range of antioxidants and micronutrients that work together to support cardiovascular health and vascular integrity.

Further more, polyherbal teas complement established a blood pressure lowering dietary approaches (such as the Mediterranean diet) by incorporating a concentrated source of functional bioactive delivery and functional formulation. For instance, a whole beverage that is formulated with polyherbal constituents to increase potassium, magnesium and polyphenols might provide greater blood pressure benefit compared to the standard diet intake alone (Lee *et al.*, 2024). Furthermore, the affordability, distinct taste, and accessibility of herbal teas can improve patient adherence to long term dietary interventions and also is an important factor in sustained cardiovascular support and management of hypertension (Hajzer *et al.*, 2025). Polyherbal tea represent a valuable adjunct in hypertension management by combining nutritional and therapeutic properties that have physiological effects on vascular function and blood pressure regulation. Their successful integration into dietary regimens alongside the daily

lifestyle, routines and pharmacologic interventions holds promise for improving cardiovascular outcomes in hypertensive populations.

2.7 ANTIOXIDANT ASSAYS IN HYPERTENSION RESEARCH (DPPH and FRAP)

To evaluate the antioxidant capacity of herbal teas, laboratory assays are often employed. Since antioxidants help to reduce oxidative stress that cause hypertension, these tests help us to understand how effective the polyherbal teas in the management or prevention of high blood pressure. Two widely used methods for this study are the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and the Ferric Reducing Antioxidant Power (FRAP) assay.

DPPH assay (Radical Scavenging Test)

This method measures the ability of plant extracts to donate hydrogen atoms or electrons to neutralize the stable DPPH free radical. The reduction in DPPH absorbance indicates antioxidant strength. A higher radical scavenging activity suggests greater ability for mitigating oxidative stress related to endothelial dysfunction and hypertension (Sharma *et al.*, 2020). It is a reliable and fast test in cardiovascular research (Adefegha *et al.*, 2022). When an antioxidant neutralizes it, the purple colour fades, the more the colour reduces the stronger the antioxidant activity. A strong result means the poly herbal tea may help reduce oxidative stress linked to high blood pressure.

FRAP assay (Reducing Power Test)

This technique assesses the reducing power of antioxidants by their ability to convert ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions in the presence of a colorimetric reagent. The increase in absorbance is proportional to antioxidant concentration. High FRAP values indicate strong electron-donating capacity of herbal extracts, which correlates with their potential role in cardiovascular protection

essential for hypertension management (Gupta *et al.*, 2021). The more the colour changes, the more powerful the antioxidant. High FRAP values indicates the polyherbal tea has strong reducing power, supporting vascular health. FRAP is commonly used in polyherbal assay because of its sensitivity and reproducibility (Rahman *et al.*, 2023).

These assays provide complementary insights, while DPPH focuses on free radical scavenging (that is, it checks how well the tea can remove harmful free radicals), FRAP highlights reducing power (that is, it checks on well the tea can reduce iron, showing it can protect cells from damage). Together, they offer a comprehensive evaluation of the antioxidant potential of polyherbal teas and help link laboratory findings to their proposed antihypertensive benefits.

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Plant Materials

- *Justicia carnea*
- *Moringa oleifera*
- *Vernonia amygdalina*
- *Gongronema latifolium*
- *Telfairia occidentalis*
- *Hibiscus sabdariffa*

3.1.2 Apparatus and Equipment

- Hand gloves
- Foil paper
- Masking taps
- Glass jar
- Spatula
- Weighing balance
- Chiffon cloth
- Laboratory sieves
- Electric blender
- Digital weighing scale

- Freeze dryer (lyophilizer)
- Electric kettle
- Ring boiler
- Refrigerator
- Rotary evaporator
- UV–Visible spectrophotometer
- Water bath
- Clean plastic bowls
- Funnels
- Filter papers
- Clean napkins
- Atomic Absorption spectrophotometer (AAS)
- Beaker
- Mechanical shaker
- Conical flask
- Pipette
- Test tube

3.1.3 Chemical and Reagents.

- DPPH reagent (2,2-diphenyl-1-picrylhydrazyl)
- FRAP reagents (Ferric Reducing Antioxidant Power reagents)
- Distilled water
- Ethanol (for extraction where necessary)

- Methanol
- Ascorbic acid standard
- Acetate buffer

3.2 METHODS

3.2.1 Collection of the selected leaves

Fresh leaves of *Justicia carnea*, *Moringa oleifera*, *Vernonia amygdalina*, *Gongronema latifolium*, *Hibiscus sabdariffa*, and *Telfairia occidentalis* were collected from local farms and some from the New Benin and Ring Road Market, Edo State, Nigeria. The leaves were examined and authenticated at the Department of Plant Biology and Biotechnology, University of Benin, Nigeria and was given an Herbarium number. This authentication process is a critical step in herbal research to avoid misidentification and ensure reproducibility of results (Akinmoladun *et al.*, 2022).

After collection, the leaves were carefully plucked out from their stems, then it was thoroughly washed to remove dirt and rinsed with distilled water while wearing hand gloves to prevent contamination of the leaves. It was then cleaned with a clean napkin. The leaves were also shade-dried at ambient temperature to preserve phytochemical constituents. Shade-drying is often preferred to sun-drying because excessive heat and ultraviolet light may degrade bioactive compounds.

After drying, the dried leaves were measured constantly for three days to get the exact constant weight. Then the dried leaves were ground into fine powder using an electric blender, and passed through a sieve to ensure uniform particle size. The powdered leaves were weighed and stored in an airtight container until further use.

3.2.2 Preparation of The Polyherbal Tea

Fifty grams (50g) of each of the powdered leaves were measured and then mixed thoroughly with 1 litre of hot distilled water at 90°C for 30 minutes to obtain a polyherbal mixture. The polyherbal tea was then carefully filtered using a two layered chiffon cloth to remove debris and this process was repeated twice to ensure the absence of residues. The filtrate was freeze dried using a lyophilizer, which helps preserve antioxidant activity by removing moisture at low temperatures without destroying heat-sensitive phytochemicals.

The dried polyherbal tea extract was stored in an airtight container under dry conditions and stored in refrigerator at -20°C until required for biochemical assays. This method of preparation is consistent with standard procedures in herbal pharmacological procedures (AOAC, 2022).

3.3 ANTIOXIDANTS ASSAYS (DPPH and FRAP)

Qualitative and quantitative phytochemical analyses were performed using standard procedures (Trease and Evans, 2002). Portion of the pulverized plant (5 g) was boiled with 20mL of distilled water gently on a water bath for 10 min. The mixture was allowed to cool and filtered. The resultant filtrate was used for the different tests.

3.3.1 DPPH Radical Scavenging Activity

Reagents; 0.3 mM DPPH solution in methanol, methanol, ascorbic acid standard, distilled water.

Procedure

The free radical scavenging capacity of the plant extracts against 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical was determined by a slightly modified method of Brand-Williams et al. Briefly, 0.5 ml of 0.3 mM DPPH solution in methanol was added to 2 mL of various

concentrations of the extracts (0.2 – 1.0 mg/ml). The test tubes were shaken and incubated in the dark for 15 minutes at room temperature, and the absorbance was read at 517 nm against methanol blank. All tests were performed in triplicate. Ascorbic acid (vitamin C) was used as control, with similar concentrations as the test samples. A blank containing 0.5 ml of 0.3 mM DPPH and 2 ml methanol was prepared and treated as the test samples.

The radical scavenging activity was calculated as shown in Equation 2:

$$\text{DPPH radical scavenging activity (\%)} = \frac{A_0 - A_1}{A_0} \times 100 \dots\dots\dots (2)$$

Where;

A_0 = absorbance of DPPH + methanol

A_1 = absorbance of DPPH + extract

3.3.2 Ferric Reducing Antioxidant Power (FRAP) Assay

Reagent: 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution, FeSO_4 standard solution, distilled water.

Procedure:

A modified method of Benzie and Strain (1996) was used for the FRAP assay. The principle behind this assay is the ability of the sample to reduce ferric tripyridyltriazine (Fe (III)- TPTZ) complex to ferrous tripyridyltriazine (Fe (II) – TPTZ), which at low pH produces an intense blue colour that can be read at 593 nm. Briefly, 1.5 ml of freshly prepared FRAP solution (25 mL of 300 mM acetate buffer pH 3.6, 2.5 ml of 10 mM 2,4,6-tripyridylstriaizine (TPTZ) in 40 mM HCl, and 2.5 ml of 20 mM ferric chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) solution) was mixed with 1 ml of varied

concentrations of the extracts (0.2 – 1.0 mg/ml). The reaction mixtures were incubated at 37°C for 30 minutes and the absorbance was read at 593 nm. Ascorbic acid served as the control, while FeSO₄ was used for calibration and values expressed as mmol FeSO₄ equivalents per gram of sample.

3.4 STATISTICAL ANALYSIS

All data regarding mineral properties were analyzed statistically using SPSS (Version 25.0). The results are expressed as the mean ± standard deviation (SD). Differences among sample means were assessed using one-way Analysis of Variance (ANOVA). Significant differences were identified using Duncan's Multiple Range Test (DMRT) at a 5% probability level ($p < 0.05$).

CHAPTER FOUR

RESULTS

4.1 DPPH RADICAL SCAVENGING ACTIVITY

The DPPH assay measures how well the extract can neutralize free radicals. The higher the percentage of inhibition, the better the antioxidant activity.

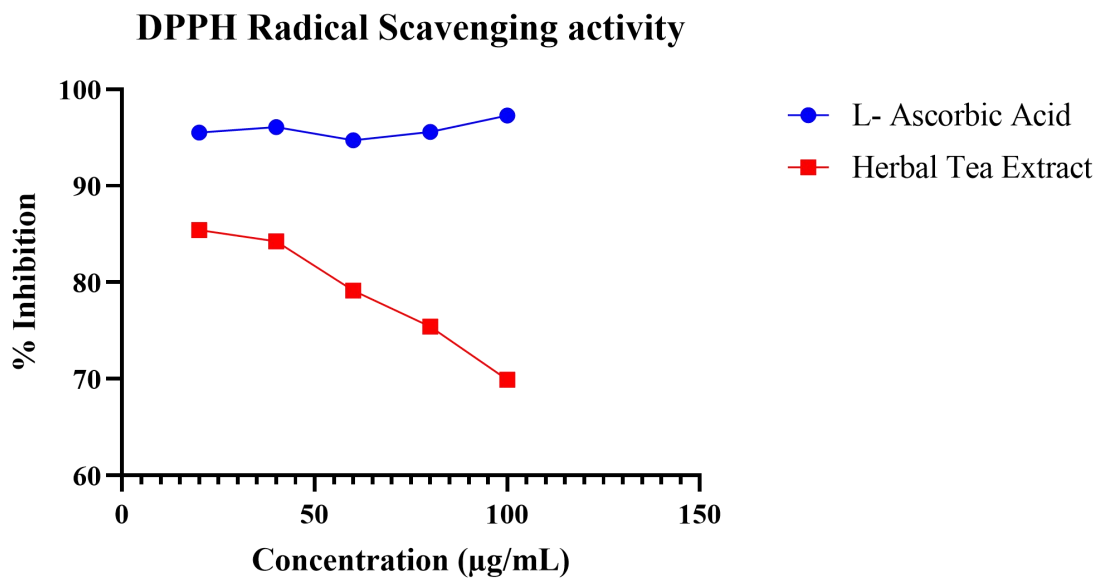


Figure 4.1: DPPH radical scavenging activity of polyherbal tea formulations

Table 4.1: DPPH Radical Scavenging Activity (IC₅₀) of Sample Formulations

Sample Formulation	IC ₅₀ Value (mg/mL)	Goodness of Fit (R ² R ²)	Activity Classification
L- Ascorbic acid	<0.001	0.14*	Standard
Herbal Extracts	0.428	0.9	Good

IC₅₀ = the concentration required to inhibit 50% of DPPH radicals.

Lower IC₅₀ = stronger antioxidant

4.2 FERRIC REDUCING ANTIOXIDANT POWER (FRAP)

The FRAP assay, measures the ability of the extracts to reduce Fe³⁺ to Fe²⁺ which is a sign of antioxidants ability.

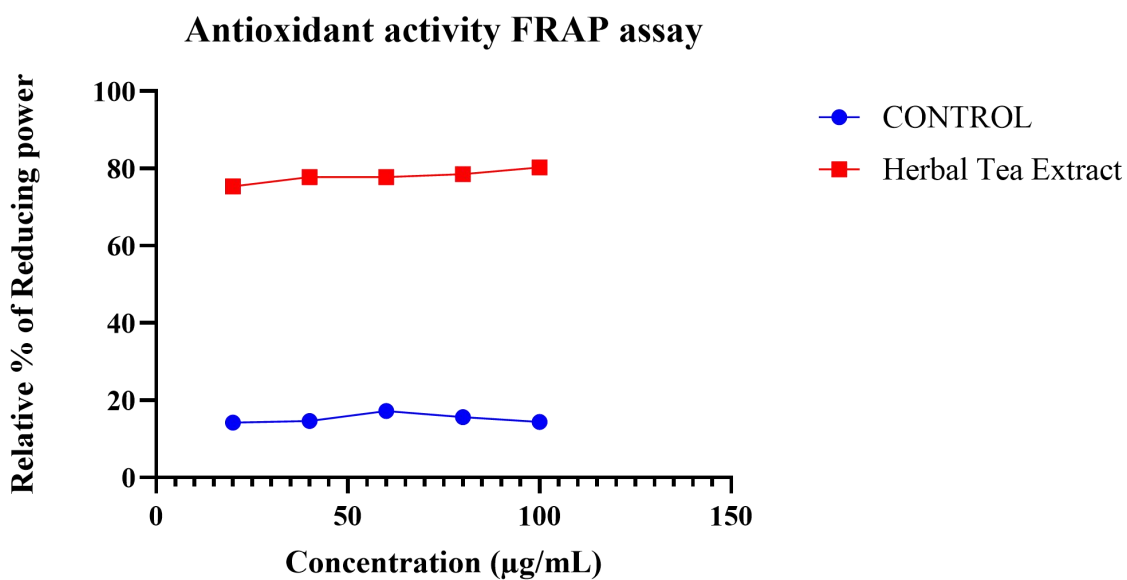


Figure 4.1: Ferric Reducing Antioxidant Power (FRAP)

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

This study evaluated the antioxidant activity of the herbal extract using two standard in-vitro assays: DPPH radical scavenging activity and Ferric Reducing Antioxidant Power (FRAP). These assays measure different mechanisms of antioxidant action, and together they provide a comprehensive understanding of the extract's ability to neutralize free radicals and donate electrons.

The DPPH results showed that the extract possessed good radical-scavenging ability, with percentage inhibition ranging from 85.40% at lower concentrations to 71.20% at the highest concentration tested. Although the scavenging effect decreased slightly with increasing concentration, the overall values remained high, indicating the presence of bioactive compounds capable of donating hydrogen atoms to stabilize DPPH radicals. When compared with the standard antioxidant (Vitamin C), which consistently recorded inhibition values above 95%, the extract displayed lower activity but still demonstrated significant antioxidant potential.

The IC_{50} value further supports these findings. The extract had an IC_{50} of 0.428 mg/ml, which falls within the range classified as good antioxidant activity. In contrast, Vitamin C presented an IC_{50} value < 0.001 mg/ml, confirming its extremely strong scavenging potency, as expected of a pure and highly efficient antioxidant. The difference in IC_{50} values reflects the natural variation between a crude extract and a purified standard compound.

The FRAP assay also demonstrated strong antioxidant potential in the herbal extract. The reducing power of the extract ranged between 80% and 88%, significantly higher than the

control, which recorded values between 18% and 22%. This indicates that the extract contains effective electron-donating molecules capable of converting Fe^{3+} to Fe^{2+} , a key characteristic of antioxidants. The results suggest the presence of phenolic compounds, flavonoids, and other phytochemicals typically associated with redox-based antioxidant activities.

5.2 CONCLUSION

This study assessed the antioxidant properties of a herbal extract using DPPH and FRAP assays. The extract demonstrated good radical-scavenging activity, with an IC₅₀ value of 0.428 mg/mL, and strong ferric-reducing ability, indicating the presence of potent antioxidant phytochemicals. Although Vitamin C showed superior activity, the extract still exhibited significant antioxidant potential. These results support the traditional use of the plant as a natural source of antioxidants and justify further investigation into its bioactive constituents. The total antioxidant capacity further confirmed the presence of potent phytochemicals capable of reducing oxidative stress, which is a major contributor to endothelial dysfunction in hypertension. These findings further support the traditional use of this polyherbal tea in managing hypertension. The observed antioxidant properties, along with its potential influence on oxidative stress pathways, indicate that it may contribute meaningfully as an adjunctive option for blood pressure control.

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APPENDICES

APPENDIX A: Plant Materials Used in the Study.

This appendix presents the plant leaves used in the formulation of the polyherbal tea for the management of hypertension.

S/N	Plant Name	Common Name	Part Used
1	<i>Justicia carnea</i>	Blood leaf	Leaf
2	<i>Moringa oleifera</i>	Moringa	Leaf
3	<i>Vernonia amygdalina</i>	Bitter leaf	Leaf
4	<i>Hibiscus sabdariffa</i>	Zobo leaf	Leaf
5	<i>Gongronema latifolium</i>	Utazi	Leaf
6	<i>Telfairia occidentalis</i>	Fluted pumpkin	Leaf

APPENDIX B: Concentration Range of DPPH and FRAP Assays

SAMPLE	DPPH			Aver				DPPH	FRAP			FRAP	×10-2
ID	Abs 1	Abs 2	Abs 3	Abs	A0	%	% Inhibition	Abs 1	Abs 2	Abs 3	Abs @ 593 nm	593 nm	
K1	0.228	0.256	0.228	0.2373	1.626	100	85.4039	0.719	0.76	0.782	0.7537	75.4	
K2	0.266	0.246	0.257	0.2563	1.626	100	84.2353	0.803	0.792	0.739	0.778	77.8	
K3	0.317	0.332	0.368	0.339	1.626	100	79.1513	0.794	0.773	0.767	0.778	77.8	
K4	0.372	0.402	0.425	0.397	1.626	100	75.4203	0.786	0.782	0.788	0.7853	78.5	
K5	0.507	0.472	0.489	0.4893	1.626	100	69.9057	0.808	0.808	0.792	0.8027	80.3	
A1 (std)	0.07	0.078	0.071	0.073	1.626	100	95.5105	0.182	0.139	0.104	0.142	14.2	
A2	0.064	0.068	0.059	0.0637	1.626	100	96.0845	0.142	0.147	0.149	0.146	14.6	
A3	0.086	0.093	0.078	0.0857	1.626	100	94.7314	0.196	0.192	0.129	0.172	17.2	
A4	0.068	0.075	0.072	0.0717	1.626	100	95.5925	0.152	0.171	0.146	0.156	15.6	
A5	0.037	0.051	0.045	0.044	1.626	100	97.2735	0.169	0.124	0.139	0.144	14.4	

Concentrations range: 20, 40, 60, 80 and 100 ug/mL