In vitro ASSESSMENT OF NITRIC OXIDE SCAVENGING ACTIVITIES AND TOTAL ANTIOXIDANT CAPACITY OF A POLYHERBAL INDIGENOUS ANTIHYPERTENSIVE TEA FORMULATION

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A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL BIOCHEMISTRY, SCHOOL OF BASIC MEDICAL SCIENCES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE, B.Sc. (HONS) MEDICAL BIOCHEMISTRY, OF THE UNIVERSITY OF BENIN, BENIN CITY.

NOVEMBER, 2025

CERTIFICATION

We the undersigned hereby certify that OMOROGIUWA ERICKSON OSA-EVBIE with Matriculation Number BMS2101444 Carried out this work titled; "*In vitro* ASSESSMENT OF NITRIC OXIDE SCAVENGING ACTIVITIES AND TOTAL ANTIOXIDANT CAPACITY OF A POLYHERBAL INDIGENOUS ANTIHYPERTENSIVE TEA FORMULATION", in the Department of Medical Biochemistry, University of Benin, Benin city and we approve same as adequate in scope and quality for the reward of Bachelors of Science Degree (B.Sc) in Medical Biochemistry.

.....… **…….……..…….....**

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(External Examiner) **DATE**

DEDICATION

This project is dedicated to Almighty God, the giver of life who has made it possible to complete my Bachelor of Science Degree (B.Sc.) program in the Department of Medical biochemistry.

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ABSTRACT

Hypertension is closely linked to oxidative stress, endothelial dysfunction, and reduced nitric oxide (NO) availability. Traditional medicinal plants are widely used for its management, yet scientific evidence supporting their efficacy remains limited. This study therefore evaluated the in vitro antihypertensive potential of a polyherbal indigenous tea prepared from *Justicia carnea* (Hospital too far), *Moringa oleifera* (miracle tree), *Vernonia amygdalina* (bitter leaf), *Gongronema latifolium* (Utazi), *Telfairia occidentalis* (fluted pumpkin), and *Hibiscus sabdariffa* (zobo). The formulation was assessed for nitric oxide scavenging activity and total antioxidant capacity (TAC), two key mechanisms involved in blood pressure regulation. Nitric oxide scavenging ability was determined using sodium nitroprusside as the NO donor, while percentage inhibition values were used to estimate the IC_{50}. Antioxidant capacity was evaluated using the phosphomolybdenum assay. The polyherbal extract demonstrated strong NO scavenging activity with an IC_{50} value of 0.313 mg/mL, indicating consistent and concentration-dependent inhibition of NO radicals. The TAC results also showed a clear increase in antioxidant activity with rising extract concentration, confirming the presence of potent bioactive compounds such as flavonoids, phenolics, tannins, and anthocyanins that can neutralize reactive oxygen species. These findings suggest that the polyherbal tea possesses significant in vitro antihypertensive potential through its ability to preserve nitric oxide and reduce oxidative stress. The study provides scientific support for the traditional use of these plants in managing hypertension and highlights the formulation as a promising complementary remedy. Further *in vivo* studies and clinical investigations are recommended to establish its safety and therapeutic applicability.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Hypertension is a leading non-communicable disease and a major contributor to cardiovascular morbidity and mortality worldwide. It is defined as a persistent rise in blood pressure above normal physiological levels and is driven by mechanisms such as activation of the renin–angiotensin–aldosterone system (RAAS), endothelial dysfunction, oxidative stress, and sympathetic overactivity (Hall *et al*., 2024). If uncontrolled, it can lead to complications such as stroke, heart failure, kidney damage, and premature death. Conventional drugs like diuretics, and calcium channel blockers are effective but limited by cost, side effects, and poor accessibility, especially in low- and middle-income countries (Carey *et al*., 2022; Tjahjono, 2024). This has renewed interest in medicinal plants, which are affordable, culturally acceptable, and rich in bioactive compounds such as flavonoids, alkaloids, and phenolics. These compounds act through diverse mechanisms, including nitric oxide mediated vasodilation (da Silva *et al*., 2021; Ahmad *et al*., 2023).

In traditional medicine, polyherbal formulations are often preferred over single plants because of their synergistic effects, broader therapeutic activity, and reduced toxicity (Kumar *et al*., 2023). Several Nigerian plants, including *Justicia carnea, Moringa oleifera, Vernonia amygdalina, Gongronema latifolium, Telfairia occidentalis,* and *Hibiscus sabdariffa*, are widely consumed and associated with cardiovascular benefits. However, their combined use as a polyherbal indigenous tea has not been fully validated, creating a need for systematic scientific evaluation.

1.2 AIM OF THE STUDY

The aim of this study was to evaluate the *in vitro* inhibitory antihypertensive potential of a polyherbal indigenous tea formulated from *Justicia carnea* (Hospital too far), *Moringa oleifera* (miracle tree), *Vernonia amygdalina* (bitter leaf), *Gongronema latifolium* (Utazi), *Telfairia occidentalis* (fluted pumpkin), and *Hibiscus sabdariffa* (zobo).

1.3 OBJECTIVES OF THE STUDY

The specific objectives of this study were to;

1. Prepare a polyherbal indigenous tea using selected plant materials.
2. Evaluate the nitric oxide scavenging capacity of the formulation.
3. To Evaluate the Total antioxidant capacity of the formulation.

1.4 JUSTIFICATION OF THE STUDY

This study is justified by the need for safe, affordable, and effective alternatives in hypertension management, especially in low-resource settings. The selected plants are culturally accepted and widely consumed in Nigeria, and each has demonstrated antihypertensive properties such as nitric oxide scavenging activity and total antioxidant capacity. However, their combined use in a polyherbal tea remains poorly validated. Scientific evaluation of this formulation is therefore necessary to provide evidence for its traditional use and to explore its potential as a complementary therapy for hypertension.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 HYPERTENSION: DEFINITION AND PATHOPHYSIOLOGY

Hypertension, or high blood pressure, is a chronic condition characterized by persistent elevation of arterial pressure beyond the normal physiological range. According to the World Health Organization (Hall *et al*., 2024), it is defined as systolic blood pressure (SBP) ≥140 mmHg and/or diastolic blood pressure (DBP) ≥90 mmHg, measured on two or more occasions. Hypertension is among the most common non-communicable diseases worldwide and a major risk factor for cardiovascular morbidity and mortality (Hall *et al*., 2024). Hypertension is classified as primary (essential), accounting for ~95% of cases, and secondary, accounting for ~5% (Iqbal and Jamal, 2023). Essential hypertension has no identifiable cause but is linked to obesity, high salt intake, family history, and genetic predisposition (Iqbal and Jamal, 2023). Secondary hypertension arises from conditions such as renal artery stenosis, chronic kidney disease, sleep apnea, and adrenal disorders (Tandan *et al*., 2023).

The disease is driven by dysregulation of mechanisms that regulate blood pressure, including the sympathetic nervous system, renin-angiotensin-aldosterone system (RAAS), endothelial dysfunction, and altered sodium and water retention (Humphrey, 2021). Increased vascular resistance due to arterial remodeling, vascular contraction, and stiffening is a hallmark feature (Humphrey, 2021). Other contributing factors include RAAS hyperactivity, overactive sympathetic drive, vasopressin, abnormal G protein-coupled receptor signaling, immune-mediated inflammation, and dysregulated vasoactive peptides (Guzik *et al*., 2024). Impaired endothelial nitric oxide synthase (eNOS) activity, oxidative stress, elevated calcium influx, and vascular smooth muscle cell (VSMC) hypertrophy further enhance vasoconstriction (Guzik *et al*., 2024). Vascular stiffness and Angiotensin II–

mediated cellular changes worsen disease progression (Humphrey, 2021). Genetic alterations affecting renal sodium handling, Na/Ca^{2+} exchange, and hormonal–neurogenic vasoconstriction have also been implicated (Humphrey, 2021). Overall, hypertension arises from increased cardiac output and systemic vascular resistance. If uncontrolled, it causes target organ damage in the heart, kidneys, brain, and vasculature. A clear understanding of its pathophysiology is crucial for designing preventive and therapeutic strategies, including the use of plant-based interventions such as polyherbal indigenous formulation (Humphrey, 2021; Guzik *et al*., 2024).

2.2 EPIDEMIOsLOGY OF HYPERTENSION

Hypertension affects more than one billion adults globally, with about 45% of the adult population living with the condition. Prevalence increases with age, affecting nearly 60% of individuals over 60 years. The World Health Organization estimates the prevalence of hypertension (BP ≥140/90 mmHg) among Nigerian adults ≥18 years at 27.8% (28.1% in men and 27.5% in women), compared to a global prevalence of 22% (Adeloye *et al*., 2021).

In adults over 25 years, prevalence rates reach 40% worldwide and 46% in Africa. A meta-analysis of 27 studies involving 27,122 Nigerians aged ≥15 years reported a pooled prevalence of 28.9% (29.5% in men, 25% in women). Mean systolic and diastolic pressures were 128.6 mmHg and 79.4 mmHg, respectively, with higher prevalence in urban (30.6%) than rural (26.4%) populations (Adeloye *et al*., 2021).

Earlier data from the 1997 Non-Communicable Diseases Survey, which used a higher diagnostic cut-off of 160/95 mmHg, reported a national prevalence of 11.2% (11.1% in men and 11.2% in women). Most studies consistently show rising prevalence with age, sex and urban–rural gradients, and an overall increasing trend over recent decades (Ayogu and Nwodo, 2021). Although ethnic and regional variations have been reported, differences in study methodologies limit direct comparisons.

2.3 DIAGNOSIS OF HYPERTENSION

Hypertension diagnosis is based on accurate blood pressure measurement. This can be performed manually using a sphygmomanometer or with automated devices for office and home monitoring. Ambulatory blood pressure monitoring is considered the most reliable, as it reduces observer bias and provides continuous readings (Ott and Schmieder, 2022).

Measurements should be taken in both arms with appropriate arm cuffs (not finger cuffs), while the patient is seated, relaxed, with legs resting on the ground and arms supported (Ruopp and Cockrill, 2022). Readings are ideally taken on an empty bladder, with at least two measurements spaced five minutes apart, repeated over two visits (Ruopp and Cockrill, 2022). In older adults, postural hypotension should also be assessed.

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.

Table 2.1: Classification of clinic blood pressure in adults (Ruopp and Cockrill, 2022)

Diagnostic category	**Systolic(mmHg)**	**Diastolic(mmHg)**
Optimal	< 120	< 80
Normal	120-129	80-84
High-normal	130-139	85-89
Grade 1 (mild) hypertension	140-159	90-99
Grade 2 (moderate) hypertension	160-179	100-109
Grade 3 (severe) hypertension	≥180	≥110
Isolated systolic hypertension	> 140	< 90

2.4 CONVENTIONAL MANAGEMENT OF HYPERTENSION

2.4.1 Non-Pharmacologic Treatment of Hypertension

Lifestyle modification plays a central role in the management of hypertension and should complement drug therapy in all patients. Key interventions include weight reduction, dietary salt restriction, regular aerobic exercise, smoking cessation, and moderation of alcohol intake (Tjahjono, 2024). These measures have been consistently shown to reduce systolic blood pressure in both hypertensive and pre-hypertensive individuals. In patients with pre-hypertension (SBP 120–139 mmHg; DBP 80–89 mmHg), lifestyle changes alone may delay or even prevent progression to established hypertension (Tjahjono, 2024). Similarly, in those with stage I hypertension (SBP 140–159 mmHg; DBP 90–99 mmHg), adherence to lifestyle modification for 6–12 months may reduce the need for pharmacologic therapy (Verma *et al*., 2021). Overall, non-pharmacologic strategies remain the foundation of hypertension management, enhancing treatment outcomes and reducing cardiovascular risk when integrated with appropriate medical therapy (Verma *et al*., 2021; Tjahjono, 2024).

2.4.2 Pharmacological Treatment of Hypertension

The choice of pharmacological therapy for hypertension is influenced by factors such as age, comorbid conditions, ethnicity, pregnancy status, and other patient-specific parameters (Carey *et al*., 2022). As such, individualized treatment regimens are required, but the major drug classes are discussed below.

Angiotensin II Receptor Blockers (ARBs) also target the RAAS by selectively blocking AT1 receptors, thereby preventing the downstream effects of angiotensin II. As their mechanism of action is similar to ACE inhibitors, patients on ARBs experience comparable cardiovascular benefits, but

without the bothersome cough associated with ACE inhibitors (Gallo *et al*., 2022). Although combining ACE inhibitors and ARBs may theoretically provide more complete RAAS blockade if blood pressure control is not achieved with monotherapy, clinical practice often favors the use of other classes, such as thiazide diuretics, in combination therapy (Alcocer *et al*., 2023)

Calcium Channel Blockers (CCBs) prevent calcium influx into vascular smooth muscle, promoting vasodilation. Dihydropyridines primarily reduce vascular resistance, whereas non-dihydropyridines also lower heart rate and contractility. CCBs have been shown to reduce all-cause mortality and stroke risk in hypertensive patients (Lee, 2023). They are often combined with ACE inhibitors or ARBs, though caution is needed when used with β-blockers or in heart failure (Lee, 2023).

Diuretics promote renal sodium and water excretion, lowering blood pressure. (Reinhart *et al*, 2023). Thiazide diuretics are widely used, with proven benefits in reducing cardiovascular events and stroke (Ernst and Fravel, 2022). Spironolactone is effective in hypertensive patients with heart failure, reducing morbidity and mortality (Taylor *et al*., 2025). Loop diuretics with potassium-sparing agents may prevent electrolyte disturbances. However, thiazides increase the risk of type II diabetes, warranting lifestyle modifications in at-risk patients (Ernst and Fravel, 2022; Reinhart *et al*, 2023).

Beta-blockers reduce cardiac output and renin release, making them preferred in patients with myocardial infarction or heart failure (Fici *et al*., 2023). Adverse effects include fatigue, sexual dysfunction, depression, and altered glucose metabolism, which can limit compliance. Caution is advised in diabetic patients due to masked hypoglycemia (Fici *et al*., 2023).

Aldosterone Antagonists, and in particular spironolactone (belonging to the mineralocorticoid receptor antagonist family), deserve special mention due to their utility when used in combination with ACE inhibitors, ARBs, CCBs, and diuretics especially in patients with heart failure. However, its use as an anti-hypertensive has been shown to be excellent in those patients that can tolerate it

(Azizi, 2021). Common problems with its use include gynaecomastia, and electrolyte disturbances such as hyperkalaemia and deteriorating renal function. The latter two are more common in patients in whom renal function is already compromised, and in patients already on ACE inhibitors or ARBs (Azizi, 2021; Gomez-Sanchez and Gomez-Sanchez, 2023; Williams, 2023).

Alpha-blocker use in treatment of hypertension continues to evolve since its inception over a decade ago. This drug, which inhibits vascular sympathetic tone by blocking postganglionic α1-receptors, is usually used as add-on medication in patients with uncontrolled hypertension or in those who have poor tolerance to other first-line medications (Kumar *et al*., 2021). Owing to its two main side effects, namely first dose syncope and vasovagal syncope, a measure of caution should be practiced during initiation of such therapy. Coupling of alpha-blockers with a diuretic can increase efficiency of therapy, particularly since it could potentially offset adverse glucose and lipid imbalances caused by diuretic therapy (Kumar *et al*., 2021). The drug is especially useful when treating hypertension in older male patients with benign prostrate hypertrophy (Kumar *et al*., 2021; Mancia and Kjeldsen, 2024).

Other drugs in the armamentarium against hypertension include direct vasodilators and centrally acting adrenergic inhibitors; however, their use in practice has diminished since their side effects outweigh benefits and they are now utilised as add-on therapy in specific patient groups. Direct renin inhibitors, which target a different part of RAAS compared to ACE inhibitors and ARBs, are another option as add-on or monotherapy in patients intolerant to first-line antihypertensive therapies (Shah *et al*., 2024). However, further studies assessing the utility of the latter group are imperative considering the ambiguity of treatment efficacy and the potential side effects in specific patient subgroups (de la Sierra and Oliveras, 2024). Other potential targets such as endothelin receptor antagonists and vasopeptidase inhibitors have been studied but no role has yet been identified for these therapies (de la Sierra and Oliveras, 2024).

2.5 ROLE OF MEDICINAL PLANTS IN HYPERTENSION MANAGEMENT

Medicinal plants have long played a central role in the prevention and treatment of hypertension across many cultures. The World Health Organization estimates that about 80% of the global population relies on herbal medicines for primary healthcare needs, particularly in low- and middle-income countries where access to modern pharmaceuticals is often limited (Basati *et al*., 2023). This dependence is not only due to affordability and accessibility but also because of the perceived safety and cultural acceptance of plant-based remedies (Basati *et al*., 2023).

The therapeutic potential of medicinal plants in the management of hypertension is largely attributed to the rich diversity of their bioactive compounds, including alkaloids, flavonoids, tannins, saponins, terpenoids and phenolic compounds (Aloufi *et al*., 2022). These phytochemicals exert blood pressure-lowering effects through multiple biological pathways, which gives medicinal plants a distinct advantage over synthetic drugs that typically act through a single mechanism (Aloufi *et al*., 2022). One of the most widely recognized pathways is the enhancement of nitric oxide bioavailability. Nitric oxide is a potent vasodilator that plays a critical role in maintaining vascular tone and blood flow (Aloufi *et al*., 2022). Many plant-derived flavonoids and phenolics stimulate nitric oxide production in the endothelium, thereby promoting vasodilation and reducing systemic vascular resistance. Another important mechanism is the inhibition of angiotensin-converting enzyme (ACE). This enzyme catalyzes the conversion of angiotensin I to angiotensin II, a powerful vasoconstrictor responsible for elevating blood pressure (Aloufi *et al*., 2022). Some plants also exert mild diuretic effects, promoting the excretion of excess sodium and water through the kidneys, leading to reduced plasma volume and lower blood pressure (Aloufi *et al*., 2022). Furthermore, phytochemicals in certain plants modulate calcium channels and relax vascular smooth muscles,

while others contribute to improved lipid metabolism and glucose regulation, thereby addressing metabolic risk factors that predispose individuals to hypertension (Verma *et al*., 2021).

Despite these promising results, the clinical application of medicinal plants is often hindered by challenges such as variability in phytochemical content, lack of standardized dosages and limited large-scale clinical trials. Nonetheless, the growing body of evidence underscores the potential of medicinal plants as either primary therapeutic agents or complementary options alongside conventional antihypertensive drugs. Their ability to act through multiple Publishing coupled with relatively fewer side effects, makes them an attractive focus for continued research, especially in the context of indigenous polyherbal formulation that combine the strengths of different plants (Basati *et al*., 2023).

2.6 POLYHERBAL INDIGENOUS FORMULATION: CONCEPT AND THERAPEUTIC ADVANTAGES

The concept of polyherbal indigenous formulation has its roots in traditional medicine, where the combination of different plants is used to achieve a more potent therapeutic effect than when a single plant is administered alone (Kumar *et al*., 2023). This approach is based on the principle of synergy, whereby the bioactive compounds present in different plants interact to enhance efficacy, minimize adverse effects, and broaden the spectrum of activity. While single herbs may contain specific phytochemicals that target a particular mechanism, combining multiple herbs provides a wide array of secondary metabolites that can act on several physiological pathways simultaneously (Kumar *et al*., 2023).

In the management of hypertension, polyherbal indigenous formulations are particularly advantageous because of the complex and multifactorial nature of the disease. Hypertension involves mechanisms such as activation of the renin–angiotensin–aldosterone system, endothelial dysfunction,

oxidative stress, sympathetic overactivity, and electrolyte imbalance (Patel *et al*., 2025). A single therapeutic agent may not sufficiently address all of these mechanisms, whereas a carefully designed polyherbal preparation offers a multi-targeted approach. For example, while one plant may function as a vasodilator through nitric oxide stimulation, another may inhibit angiotensin-converting enzyme (ACE), and yet another may provide antioxidant protection or diuretic effects. The combined action of these plants therefore offers a more comprehensive means of controlling blood pressure and reducing the risk of target organ damage (Patel *et al*., 2025).

Another important advantage of polyherbal indigenous formulations lies in their ability to reduce side effects. The combination of different herbs at lower individual doses may lower the likelihood of toxicity that might occur if higher doses of a single plant extract were used (Aladejana, 2023). Furthermore, some phytochemicals present in one plant may counteract or mitigate the potential adverse effects of another, thereby improving overall safety and tolerability. This balance between efficacy and safety is one of the key reasons why polyherbal therapies have been widely accepted in indigenous healthcare systems (Aladejana, 2023).

2.7 OVERVIEW OF SELECTED PLANTS USED IN THE POLYHERBAL INDIGENOUS TEA

The polyherbal indigenous formulation investigated in this study was developed using six indigenous plants traditionally consumed as vegetables, spices, or herbal remedies in Nigeria. These plants were selected based on their reported nutritional and therapeutic values, particularly their roles in cardiovascular health. A concise overview of each plant is presented below.

2.7.1 *Justicia carnea*

Justicia carnea, a species of the genus Justicia in the family Acanthaceae, is a perennial herbaceous plant commonly referred to as Jacobinia, Flamingo plant, or “Hospital too far.” Morphologically, *Justicia carnea* grows to a height of 3–6 feet with evergreen leaves and a highly branched stem. The leaves are simple, opposite, elliptic, and acute, with entire margins, pinnate venation, and a deciduous nature. Its inflorescence is a spike bearing clustered, fragrant flowers that may appear in white, pink, apricot, yellow, or lavender colors, reflecting varietal differences within the species. The fruit is brown, pod-like in structure, and characterized by a dry, hard surface (Asiwe *et al*., 2023).

The medicinal importance of *Justicia carnea* is well recognized, particularly in southern Nigeria, where it is widely utilized in traditional medicine. Contemporary research has demonstrated that the plant possesses anti-inflammatory, antimicrobial, hepatoprotective, and anticancer properties. In ethnomedicine, decoctions of *Justicia carnea* are employed to boost blood levels, aid digestion, manage dysentery, enhance immunity, and treat influenza and diarrhea (Asiwe *et al*., 2023; Okocha *et al*., 2023).

Phytochemical studies have revealed that *Justicia carnea* contains alkaloids, flavonoids, saponins, tannins, and glycosides, which contribute to its pharmacological activities. Pharmacologically, the plant has been reported to exhibit antioxidant, anti-inflammatory, and hematopoietic effects, making it particularly relevant in the context of hypertension management by mitigating oxidative stress and improving vascular health (Asiwe *et al*., 2023).

Figure 2.1: *Justicia carnea* (Asiwe *et al*., 2023)

2.7.2 *Moringa oleifera*

Moringa oleifera, belonging to the family Moringaceae, is a fast-growing, perennial plant widely acclaimed for its nutritional and medicinal value. It is popularly referred to as the "wonder tree" or "miracle tree" because nearly every part of the plant is useful for food, medicine, or industrial purposes. Although *Moringa oleifera* is indigenous to the sub-Himalayan tracts of India, Pakistan, Bangladesh, and Afghanistan, where it has long been employed in folk medicine. it has now become naturalized and cultivated throughout tropical and subtropical regions worldwide (Islam *et al*., 2021).

Nutritionally, the leaves of *Moringa oleifera* are rich in essential vitamins, including vitamin A, vitamin C, and vitamin E, and are also excellent sources of minerals such as calcium, potassium, and magnesium. In addition, phytochemical investigations have identified significant quantities of flavonoids, phenolic acids, tannins, saponins, alkaloids, and glucosinolates, which contribute to its wide array of biological activities (Gharsallah *et al*., 2023).

Traditionally, *Moringa oleifera* has been used to manage hypertension, diabetes, inflammation, infections, malnutrition, and gastrointestinal disorders. In many communities, decoctions and leaf extracts are consumed as affordable remedies to enhance general well-being and nutritional status. Its role in combating malnutrition, particularly in children and pregnant women, has made it a plant of global nutritional interest (Pareek *et al*., 2023).

Scientific studies have provided evidence supporting the antihypertensive properties of *Moringa oleifera*. These effects are mediated through several mechanisms, including vasodilation, diuretic action, antioxidant effects, and inhibition of angiotensin-converting enzyme (ACE) activity (Pareek *et al*., 2023).

Figure 2.2: Image of *Moringa oleifera* (Pareek *et al*., 2023)

2.7.3 *Vernonia amygdalina*

Vernonia amygdalina (family: Asteraceae), commonly known as “bitter leaf,” is a perennial shrub indigenous to tropical Africa and widely cultivated across sub-Saharan regions (Degu *et al*., 2024). It is one of the most commonly utilized medicinal plants in the genus Vernonia, highly valued for both nutritional and therapeutic purposes. The leaves are characteristically bitter due to the presence of sesquiterpene lactones, while other important phytochemicals include flavonoids, alkaloids, tannins, saponins, and glycosides, all of which contribute to its diverse biological activities (Degu *et al*., 2024).

Traditionally, decoctions and infusions of *Vernonia amygdalina* leaves are employed in the management of malaria, gastrointestinal disorders, intestinal helminth infections, and hypertension. In many African communities, the leaves are also consumed as vegetables, providing essential nutrients alongside their medicinal value (Degu *et al*., 2024).

Pharmacological studies have validated several of its folkloric uses. Extracts of *Vernonia amygdalina* have been shown to possess hypoglycemic, antihyperlipidemic, and antihypertensive effects, which are attributed to improvements in endothelial function, modulation of lipid metabolism, enhancement of insulin sensitivity, and antioxidant activity (Ugbogu *et al*., 2021). Its antioxidant properties, in particular, help reduce oxidative stress, a key factor in vascular dysfunction and hypertension development. Additionally, its antimicrobial and anti-inflammatory actions broaden its therapeutic relevance beyond cardiovascular health (Ugbogu *et al*., 2021).

Figure 2.3: *Vernonia amygdalina (*Degu *et al*., 2024)

2.7.4 *Gongronema latifolium*

Gongronema latifolium, belonging to the family Asclepiadaceae, is a widely consumed edible and medicinal plant indigenous to Nigeria and other tropical African regions (Amrelia, 2022). It thrives predominantly in rainforest zones and is recognized for its production of white latex and yellow flowers, with propagation typically achieved through seeds or stem cuttings (Edim *et al*., 2012). The plant bears various indigenous names, reflecting its broad cultural acceptance: the Ikales of Ondo State call it Iteji, the Ibos refer to it as Utazi, the Efik/Ibibio as Utasi, while the Yorubas call it Arokeke (Amrelia, 2022).

Culinarily, the leaves of *Gongronema latifolium* are widely used in soups, stews, and herbal teas, serving both nutritional and medicinal purposes. In ethnomedicine, it has long been applied in the management of hypertension, diabetes, loss of appetite, and general body weakness. Its broad use as both food and medicine underscores its classification as a functional plant with significant health benefits (Amrelia, 2022). Phytochemical studies have identified the presence of alkaloids, flavonoids, tannins, saponins, and terpenoids, which confer a wide range of pharmacological activities (Okochi *et al*., 2024). Pharmacological investigations indicate that *Gongronema latifolium* demonstrates antihypertensive effects through mechanisms such as vasorelaxation, inhibition of vascular smooth muscle contraction, and reduction of oxidative stress (Okochi *et al*., 2024). These properties are supported by its antioxidant potential, which helps to mitigate free radical damage implicated in vascular dysfunction and cardiovascular disease progression (Okochi *et al*., 2024).

Figure 2.4: *Gongronema latifolium* (Amrelia, 2022)

2.7.5 *Telfairia occidentalis*

Telfairia occidentalis, commonly known as fluted pumpkin, belongs to the family Cucurbitaceae. It is native to West Africa and predominantly cultivated in Sierra Leone, Ghana, and Nigeria. In Southern Nigeria, where it is one of the major vegetable crops, it is widely recognized by various indigenous names such as “iroko” or “apiroko” in Yoruba, “ubong” in Efik, “ugu” in Igbo, “umeke” in Edo, and “umee” in Urhobo (Ojimelukwe, 2022). Botanically, *Telfairia occidentalis* is a dioecious, perennial tropical vine cultivated for both its leaves and edible seeds (Ojimelukwe, 2022). The plant is a creeping herbaceous vegetable with lobed leaves and twisted tendrils extending over the soil surface. Fluted pumpkin can be cultivated on mounds or flatlands and is frequently grown in domestic gardens beside fences or near trees, allowing the fruits to hang from branches. It is also commonly cultivated along bamboo or wooden trellises (Ojimelukwe, 2022).

Nutritionally, *Telfairia occidentalis* leaves are rich in essential compounds and are valued for their curative, industrial, and nutritional significance. Phytochemical screening has revealed the presence of saponins, alkaloids, tannins, and phenolic compounds, confirming its medicinal potential (Akpasi *et al*., 2023). The seeds are equally valuable and can be ground and incorporated into soups, or alternatively roasted, cooked, and consumed directly. Nutritional analysis shows that the seeds contain about 45% fat, 23% carbohydrates, 20.5% protein, 2.2% fiber, and 4.8% total ash (Akpasi *et al*., 2023). Additionally, the seeds are composed of phospholipids (58%), glycolipids (26%), and neutral lipids (15%), with the seed oil comprising up to 61% unsaturated fatty acids (Akpasi *et al*., 2023).

In traditional Nigerian medicine, *Telfairia occidentalis* is highly regarded for its healing properties, which are frequently applied in the management of various ailments. Scientific studies have further supported these ethnomedicinal claims, highlighting its antioxidant and hematopoietic activities,

which contribute to its therapeutic role, particularly in cardiovascular protection and blood health. Thus, *Telfairia occidentalis* stands out as both a staple vegetable and a functional medicinal plant with wide ethnopharmacological applications (Enin *et al*., 2024; Magnus *et al*., 2024).

Figure 2.5: *Telfairia occidentalis* (Ojimelukwe, 2022)

2.7.6 *Hibiscus sabdariffa*

Hibiscus sabdariffa L. (family: Malvaceae), commonly known as “roselle” or “zobo,” is an annual shrub widely cultivated in tropical and subtropical regions. It is characterized by red or green stems, serrated leaves, and yellow flowers with a dark center. The fleshy red calyces are the most economically important part, used in beverages, foods, and traditional medicine, while the leaves, seeds, and fibers also have nutritional and industrial applications (Onyeukwu *et al*., 2023). Traditionally, *Hibiscus sabdariffa* is used as a diuretic, mild laxative, antimicrobial, and antihypertensive agent. In West Africa, especially Nigeria, “zobo” drink prepared from the calyces is popular both as a refreshment and as a natural remedy for high blood pressure and cholesterol (Onyeukwu *et al*., 2023).

Phytochemically, the calyces contain anthocyanins, flavonoids, phenolic acids, ascorbic acid, and organic acids, which contribute to their sour taste and pharmacological effects. Anthocyanins, in particular, are responsible for the red coloration and strong antioxidant activity, while hibiscus acid, protocatechuic acid, and quercetin derivatives add to its therapeutic potential (Riaz and Chopra, 2018). Scientific evidence strongly supports its antihypertensive activity, with studies showing its ability to inhibit angiotensin-converting enzyme (ACE), enhance nitric oxide–mediated vasodilation, and improve vascular reactivity (Montalvo-González *et al*., 2022). Clinical trials have reported significant reductions in systolic and diastolic blood pressure among hypertensive and pre-hypertensive patients consuming hibiscus tea or extracts. In addition, *Hibiscus sabdariffa* exhibits anti-hyperlipidaemic, antioxidant, hepatoprotective, nephroprotective, anti-inflammatory, and hematopoietic properties (Montalvo-González *et al*., 2022).

Figure 2.6: *Hibiscus sabdariffa* (Onyeukwu *et al*., 2023)

2.8 ROLE OF NITRIC OXIDE IN HYPERTENSION

Nitric oxide (NO) is recognized as one of the most important molecules produced by the body. It was named “Molecule of the Year” by Science in 1992, and in 1998, the Nobel Prize in Physiology or Medicine was awarded to the three U.S. scientists responsible for its discovery. Nitric oxide is a messenger molecule that plays a role in nearly every organ system in the body, regulating hormone secretion, nutrient metabolism, cardiac contractility, immune function, and cognitive health. One of NO’s most recognized functions is serving as a vasodilator to promote healthy blood pressure and blood flow (da Silva *et al*., 2021).

Nitric oxide (NO) is a critical regulator of vascular tone and blood pressure. It is produced from L-arginine by endothelial nitric oxide synthase (eNOS) and mediates vasodilation through activation of soluble guanylate cyclase (sGC), leading to cyclic guanosine monophosphate (cGMP) formation and relaxation of vascular smooth muscle (da Silva *et al*., 2021). Reduced plasma NO levels and impaired endothelium-dependent vasodilation have been consistently reported in patients with essential hypertension (da Silva *et al*., 2021). Strategies to enhance NO bioavailability include both non-pharmacological and pharmacological approaches. Physical exercise has been shown to improve endothelial function and NO levels (Bryan, 2022; da Silva *et al*., 2021), while supplementation with L-arginine or cofactors such as tetrahydrobiopterin can restore eNOS activity. L-arginine supplementation is widely studied as a means of enhancing NO production. By serving as a substrate for eNOS, it promotes vasodilation and blood pressure reduction (Bryan, 2022). The upregulation of the NO/sGC/cGMP pathway in arterial hypertension has been identified as a promising therapeutic goal for lowering blood pressure and reducing associated complications related to heart and kidney function, based on experimental models of hypertension and NOS inhibition without causing tolerance (Bryan, 2022; da Silva *et al*., 2021).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Plant Materials

1. *Justicia carnea*
2. *Moringa oleifera*
3. *Vernonia amygdalina*
4. *Gongronema latifolium*
5. *Telfairia occidentalis*
6. *Hibiscus sabdariffa*

3.1.2 Apparatus and Equipment

1. Electric blender (for grinding dried plant leaves)
2. Digital weighing balance
3. Freeze dryer (lyophilizer)
4. Hot water kettle / ring boiler
5. Rotary evaporator
6. UV–Visible spectrophotometer
7. Water bath
8. Incubator
9. Electric blender
10. Clean plastic bowls

11. Laboratory sieves

12. Analytical weighing balance

13. Funnels

14. Filter papers

15. Glass jar with lid

16. Lyophilizer

17. Clean napkins

18. Atomic Absorption spectrophotometer (AAS)

19. Spatula

20. Masking tapes

21. Beaker

22. Mechanical shaker

23. Conical flask

24. Pipette

25. Test tube

26. Hot water bath.

3.1.3 Chemicals and Reagents

1. Griess reagent components for nitric oxide assay (sulfanilamide, phosphoric acid, N-(1-naphthyl) ethylenediamine dihydrochloride)
2. Sodium nitroprusside (NO donor)
3. Angiotensin-converting enzyme (ACE) from rabbit lung
4. Hippuryl-histidyl-leucine (HHL) substrate
5. Distilled Water
6. Tris–HCl buffer
7. Ethanol (for extraction where necessary)

3.2 COLLECTION AND PREPARATION OF POLYHERBAL TEA

3.2.2 Collection and Authentication

Fresh leaves of all selected plants were collected from local gardens and markets in Benin City, Edo State, Nigeria, and authenticated at the Department of Plant Biology and Biotechnology, University of Benin. Authentication was carried out by a plant taxonomist to ensure correct botanical identity. Leaves of all selected plants were plucked from healthy stems, washed thoroughly to remove dust and debris, and rinsed with distilled water to prevent contamination. Shade-drying was carried for four weeks out at room temperature to prevent degradation of heat-sensitive phytochemicals. Leaves were weighed daily until constant weight was achieved.

Dried leaves were ground into fine powder using an electric blender. The powders were passed through a fine mesh sieve to ensure uniform particle size. Each powdered plant material was stored in airtight jars under dry conditions.

3.2.3 Preparation of the Polyherbal Tea mixture

Each leaf powder (50g) was steeped in 1L of hot distilled water (90°C) for 30 minutes while being stirred. After cooling the filtrate was collected using a filter paper and used for further analysis while the residue was discarded. Disposable hand gloves were used during sample handling.

The filtrate was lyophilized (freeze -dried) and extracts were then stored at -20°C until use.

3.3 EXPERIMENTAL ASSAYS

3.3.1 Total Antioxidant Capacity (TAC)

The TAC of the extracts was evaluated using the phosphomolybdenum method based on the procedure described by Prieto *et al*. (1999). The assay is based on the reduction of Mo (+6) to Mo (+5) by the extracts and subsequent formation of green phosphate Mo (+5) complex at acidic pH. Briefly, 0.3 mL of graded concentrations of extracts was mixed with 3 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The absorbance of the reaction mixture was measured at 695 nm using a spectrophotometer against a blank after cooling to room temperature. Methanol (0.3 mL) in the place of extract was used as the blank. The TAC was expressed as milligram equivalents of ascorbic acid and calculated as shown in Equation 16:

$$\text{TAC (mg AAE/g extract)} = \frac{C \times V}{m} \quad \ldots\ldots(16)$$

where c = concentration of ascorbic acid in mg/mL extrapolated from the standard calibration curve; V = volume of extract in mL; and m = weight of crude plant extract in grams.

3.3.2 Nitric Oxide Radical Scavenging Capacity

The method described by Makhija *et al*. (2011) was used. Briefly, 1 mL of 10 mM sodium nitroprusside was mixed with 1 mL of extract prepared in phosphate buffer. The mixture was incubated at 25 °C for 150 min. To 1 mL of the incubated solution, 1 mL of Griess' reagent was added. Then the absorbance was read at 546 nm. The % inhibition of nitric oxide radical was calculated as shown in Equation 17:

$$\text{Nitric oxide scavenging activity (\%)} = \frac{A_{control} - A_{extract}}{A_{control}} \times 100 \quad \ldots\ldots (17)$$

CHAPTER FOUR

4.0 RESULTS

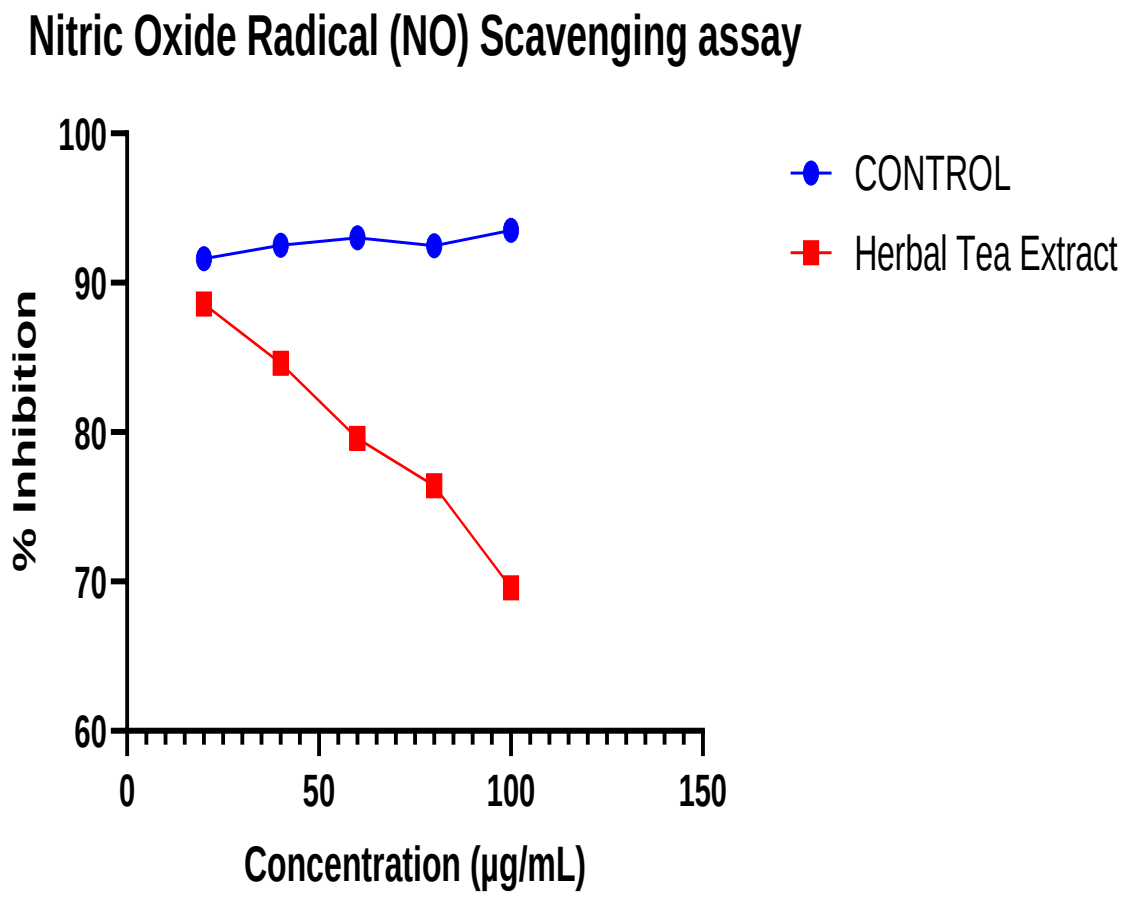

Figure 4.1: Nitric Oxide (NO) Scavenging Activity of Polyherbal Extract

Table 4.1: Nitric Oxide (NO) Scavenging Activity (IC50) of Sample Formulations

Sample	IC50 Value (mg/mL)	Interpretation
Control	< 0.001	Standard
Herbal Tea Extract	0.313	Good

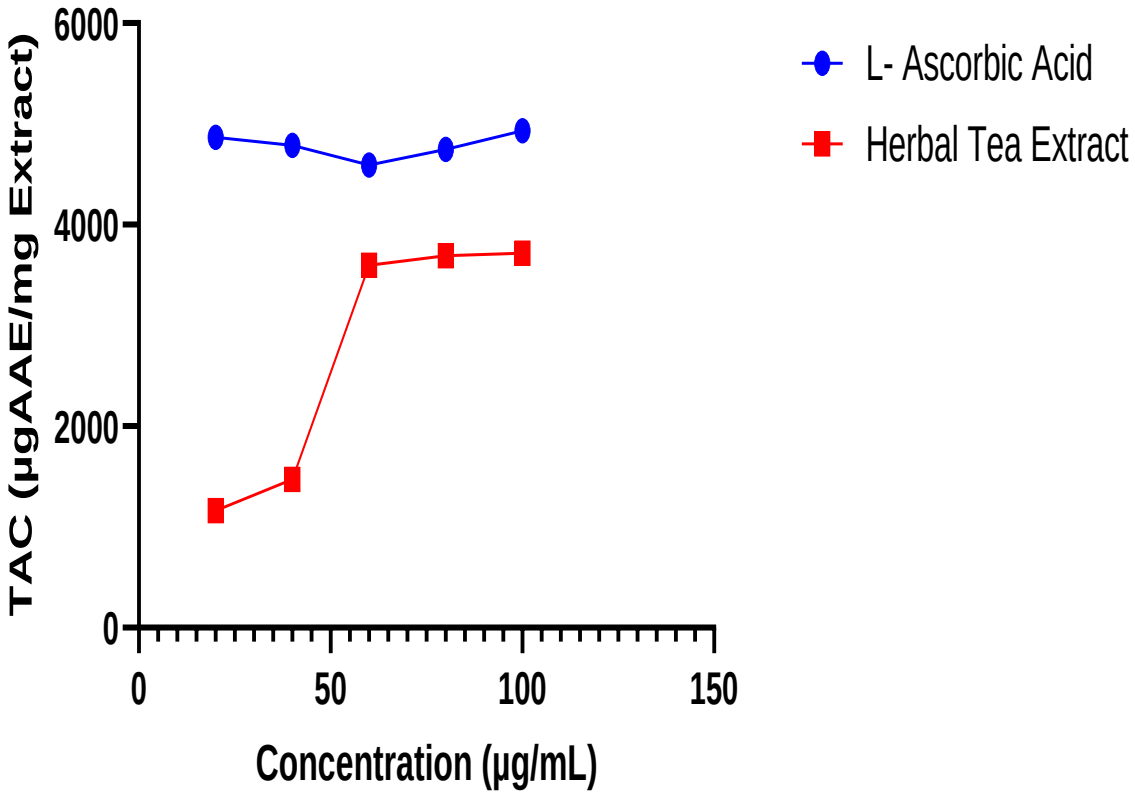

Figure 4.2: Total Antioxidant Capacity (TAC) of Polyherbal Extract

CHAPTER FIVE

5.0 DISCUSSION

The findings obtained from the nitric oxide scavenging assay and the total antioxidant capacity offer important insights into the possible therapeutic role of this formulation in the management of hypertension. The numerical values presented in Table 4.1, together with the patterns observed in the graphical representations in Chapter Four, provide a clear understanding of the biochemical behavior of the extract.

The nitric oxide scavenging assay from table 4.1 revealed that the polyherbal tea extract had an IC_{50} value of 0.313 mg/mL. This result shows that a concentration of 0.313 mg/mL of the extract was required to inhibit 50% of the nitric oxide radicals generated in the reaction mixture. In nitric oxide assays, a lower IC_{50} value indicates stronger radical scavenging activity, because it means less concentration is required to achieve 50% inhibition. Although the control had an IC_{50} value of less than 0.001 mg/mL, which represents the baseline reference inhibition, it does not serve as a competing extract but rather establishes the assay's sensitivity. The shape of the nitric oxide scavenging curve from figure 4.1 in the graph supports this reliability. The graph showed a steady increase in percentage inhibition with increasing extract concentration, meaning that as more extract was introduced, more nitric oxide radicals were neutralized. The point where the curve crossed the 50% inhibition mark corresponded to approximately 0.313 mg/mL, confirming the IC_{50} calculated from the table. This gradual, concentration-dependent inhibition is characteristic of extracts rich in flavonoids, phenolics, anthocyanins and other bioactive molecules capable of donating hydrogen atoms and electrons to neutralize free radicals (da Silva *et al*., 2021). Since nitric oxide plays a central role in vasodilation, the ability of the extract to prevent its oxidative depletion suggests that the polyherbal tea may help maintain endothelial nitric oxide availability, an essential factor in blood

pressure regulation (Verma *et al*., 2021). The biochemical relevance of this scavenging activity is particularly important in the context of hypertension. In hypertensive states, nitric oxide levels are often reduced due to increased oxidative stress and endothelial dysfunction. Free radicals readily react with nitric oxide, converting it to harmful species such as peroxynitrite, thereby impairing vasodilation. By scavenging excess radicals and protecting nitric oxide molecules, the polyherbal formulation may indirectly enhance vasodilation, improve endothelial function and reduce vascular resistance (da Silva *et al*., 2021; Bryan, 2022).

The total antioxidant capacity from figure 4.2 further strengthens the antihypertensive potential of the formulation. Although the TAC results were presented graphically rather than in table form, the graph clearly demonstrated a pronounced increase in antioxidant capacity with increasing concentration of the extract. This indicates that the formulation contains strong reducing agents capable of converting molybdenum (VI) to molybdenum (V), which is the basis of the phosphomolybdenum assay (Prieto *et al*., 1999). The upward trend seen in the TAC graph reflects a robust antioxidant response, showing that even at lower concentrations, the extract exhibited measurable antioxidant activity, which intensified progressively with higher concentrations.

Taken together, the results suggest a multi-mechanistic mode of action for the polyherbal formulation. Its antioxidant capacity counteracts oxidative stress (Hartanti, and Hamad, 2023), while its nitric oxide scavenging activity helps maintain vasodilation pathways (da Silva *et al*., 2021). Such dual action reflects the strength of polyherbal formulations, which combine bioactive compounds from multiple plants to produce synergistic therapeutic effects that are often more potent than single-plant extracts (Kumar *et al*., 2023). The relatively low IC_{50} value of 0.313 mg/mL, the strong regression coefficient, and the upward trajectory of the antioxidant capacity graph all indicate that this formulation possesses significant biochemical properties relevant to reducing high blood pressure.

5.1 CONCLUSION

This study has successfully demonstrated that the polyherbal indigenous tea formulation possesses significant *in vitro* antihypertensive properties. The formulation exhibits strong nitric oxide scavenging activity, with an IC_{50} value of 0.313 mg/mL and a strong regression fit, indicating that the formulation can effectively preserve nitric oxide availability—an essential factor for vasodilation and healthy blood pressure regulation. 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 provide a strong scientific rationale for the traditional use of this polyherbal tea in the management of hypertension. Its combined antioxidant and nitric oxide–modulating effects suggest that it may serve as a beneficial complementary therapy.

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