Vascular Reactivity Effect of the Ethanol Leaves Extract of *Alstonia boonei* on Isolated Thoracic Aorta of Wistar Rats

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A PROJECT SUBMITTED TO THE DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY, FACULTY OF PHARMACY, UNIVERSITY OF BENIN.

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CERTIFICATION

This is to certify that the project titled “vasorelaxant effect of the ethanolic extract of *Alstonia boonei* leaves on isolated thoracic aorta of Wistar rats” was carried out by Stanley Ezinna UGWU [PHA1908608] in the Department Of Pharmacology And Toxicology, Faculty Of Pharmacy, University Of Benin, Benin City, Edo State, under the supervision of Dr F.C. Amaechina. This work is original and has not been submitted elsewhere for the award of any degree.

Dr. F.C. AMAECHINA Date

(Project Supervisor)

DR. P.A UCHENDU Date

(Head of Department)

DEDICATION

I dedicate this project work to Almighty God, whose grace, wisdom, and strength made its successful completion possible, and to my mummy, whose love, prayers, and the beautiful values she instilled in me continue to guide and inspire my journey even in her absence.

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ABSTRACT

Alstonia boonei has long been used in traditional medicine on various health conditions such as, cardiovascular conditions, though its precise vasorelaxant mechanism remains unclear. Hypertension, often linked to vascular smooth muscle hypercontraction and endothelial dysfunction, is a leading cardiovascular risk. While conventional antihypertensives target these pathways, they can have side effects, high costs, and limited accessibility, prompting interest in medicinal plants as alternative therapies. This study evaluated the vasorelaxant effect of *Alstonia boonei* extract on rat thoracic aorta rings precontracted with KCl and norepinephrine. Thoracic aortae were isolated from Wistar rats and sectioned into 3–4 mm rings, mounted in a 25 mL organ bath at 37°C with continuous aeration (95% O_2, 5% CO_2), and equilibrated under 1 g resting tension. Contractions were induced with 80 mM KCl or 1 µM norepinephrine to establish baselines. The extract was then administered cumulatively, and vascular responses were recorded via PowerLab. Relaxation was expressed as a percentage of the initial contraction, with data analyzed using GraphPad Prism (mean ± SEM, $P < 0.05$). The extract induced concentration-dependent relaxation in aortic rings precontracted with both KCl and norepinephrine, suggesting inhibition of voltage-dependent calcium channels and attenuation of receptor-mediated contraction. These findings support the vasorelaxant potential of *Alstonia boonei* and provide a mechanistic rationale for its ethnomedicinal use in hypertension. The study highlights its promise as a natural source of vasorelaxant compounds, though further work is needed to isolate active constituents, elucidate molecular pathways, and assess long-term therapeutic effect

CHAPTER ONE

INTRODUCTION

1.1.0 THE CARDIAC AND THE CIRCULATORY SYSTEM

One of the human body's most vital and intricate systems is the cardiovascular system. It is made up of the heart, blood vessels, and blood itself, and it works in tandem to remove waste materials from the body's tissues and supply oxygen and nutrition. The maintenance of internal equilibrium and the continuation of life depend heavily on this system. The pulmonary and systemic circulations are the two primary circuits that make up the cardiovascular system. Deoxygenated blood is transported from the right side of the heart to the lungs via the pulmonary circuit, where it is oxygenated and then returned to the left side. After then, the systemic circuit distributes this oxygen-rich blood throughout the body, giving cells the nutrients, they require for life and metabolism (Tortora and Derrickson, 2020).

1.1.2 ANATOMY AND PHYSIOLOGY OF THE CARDIOVASCULAR SYSTEM

The maintenance of the human body's interior environment depends on the cardiovascular system. It is in charge of delivering waste materials, nutrients, hormones, and oxygen to and from tissues. The heart, blood arteries, and blood itself are the three primary parts of this system. Anatomically, these structures are arranged to facilitate constant circulation, and physiologically, they function to adapt to the body's changing needs. The heart is a muscular organ situated between the lungs in the thoracic cavity. Two ventricles and two atria make up its four chambers. While the left side of the heart receives oxygenated blood from the lungs and circulates it throughout the body via the systemic circuit, the right side of the heart receives deoxygenated blood and pumps it to the lungs via the pulmonary circuit. Electrical signals that start in the sinoatrial node and go through the conduction system coordinate the contraction of the myocardium, and valves inside the heart stop backflow (Tortora and Derrickson, 2014; Hall and Guyton, 2011).

An intricate system of arteries, veins, and capillaries is made up of the blood vessels. While veins return blood to the heart at lesser pressure, arteries carry blood away from the heart under high pressure. The blood and bodily tissues exchange gasses, nutrition, and waste materials through capillaries, which have thin walls. The tiny branches that enter and exit capillary beds, known as arterioles and venules, aid in controlling local blood pressure and flow (Silverthorn, 2010).

Blood flow and the control of vascular pressure and resistance are at the heart of the cardiovascular system's physiology. Vasodilation, or the relaxation and enlargement of blood vessels, is a crucial component of vascular physiology. Increased blood flow and reduced resistance are made possible by this mechanism, especially while exercising or reacting to certain chemical cues. Vasodilators are chemicals that cause the smooth muscle in blood vessel walls to relax. Vascular endothelium, the inner lining of blood arteries, is one of the most important locations for the synthesis of vasodilators. Nitric oxide is a strong vasodilator released by endothelial cells that relaxes nearby smooth muscle cells. The enzyme nitric oxide synthase produces nitric oxide from the amino acid L-arginine, particularly when shear stress or pharmacological stimulus is present (Moncada and Higgs, 1993). Endothelial cells also emit prostacyclin, a vasodilator that causes relaxation by raising cyclic AMP in smooth muscle cells, in addition to nitric oxide. Vasodilation, especially in smaller arteries, is facilitated by a different class of chemicals called endothelial-derived hyperpolarizing factors (Rang *et al*., 2012). At the local level, these compounds allow for precise regulation of blood flow. Vasodilators are also released by other tissues. Nitric oxide is released by the endothelial cells that line the coronary arteries in the heart to control coronary circulation. Adenosine, carbon dioxide, potassium ions, and lactic acid are examples of local vasodilators that build up in skeletal muscles during exercise and induce vasodilation to enhance blood flow and oxygen delivery (Widmaier, Raff, and Strang, 2008). These effects, which are mostly local, aid in coordinating blood flow with metabolic processes. Vasodilator release is also influenced by the autonomic nervous system. Although sympathetic stimulation typically results in vasoconstriction, some sympathetic nerves have the ability to release acetylcholine, which induces the endothelium to produce more nitric oxide and cause vasodilation. In certain vascular beds, such the skin and genitalia, this is especially noticeable (Guyton and Hall, 2006).

1.1.3 THE STRUCTURE OF THE HEART.

The heart is a muscular organ that serves as the principal pump of the cardiovascular system. Located within the mediastinum of the thoracic cavity, it lies slightly tilted to the left and is enclosed by the pericardium, a double-layered protective membrane. This sac comprises an outer fibrous layer and an inner serous layer, both of which help stabilize the heart's position and minimize friction during cardiac activity (Tortora and Derrickson, 2014). Reflecting its essential role in sustaining circulation, the heart typically weighs between 250–300 grams in adult females and 300–350 grams in adult males (Hall and Guyton, 2011). Structurally, it contains four chambers—two superior atria and two inferior ventricles. Deoxygenated blood enters the right atrium through the superior and inferior venae cavae and the coronary sinus, while oxygenated blood is delivered to the left atrium via the pulmonary veins. Each atrium directs blood into its corresponding ventricle through an atrioventricular valve. The right ventricle transports blood to the lungs through the pulmonary artery, whereas the left ventricle propels blood into the aorta for systemic circulation (Hall and Guyton, 2011). The heart wall consists of three distinct layers. The innermost endocardium lines the chambers and valves, providing a smooth surface that reduces friction and promotes efficient blood flow. The myocardium, the thick middle layer, is composed of cardiac muscle and is responsible for generating the force required for contraction; it is especially thick in the left ventricle due to its role in systemic pumping. The outermost layer, the epicardium, forms part of the pericardium and contains coronary vessels, adipose tissue, and connective structures (Silverthorn, 2010). The heart's valve system ensures unidirectional flow. The tricuspid valve separates the right atrium and ventricle, while the mitral (bicuspid) valve lies between the left atrium and ventricle. The semilunar valves—the pulmonary valve at the right ventricular outflow tract and the aortic valve at the left—prevent backflow by opening and closing in response to pressure changes throughout the cardiac cycle (McKinley, O'Loughlin, and *et al*, 2020). Internally, the atria contain pectinate muscles, and the ventricles feature trabeculae carneae, both of which contribute structural support during contraction. The heart is further organized by the interatrial and interventricular septa, which separate the right and left chambers. Embedded within these septal regions is the cardiac conduction system, comprising the sinoatrial node, atrioventricular node, bundle of His, and Purkinje fibres. These specialized components generate and propagate the electrical signals required for synchronized cardiac contraction (Widmaier, Raff, and Strang, 2008).

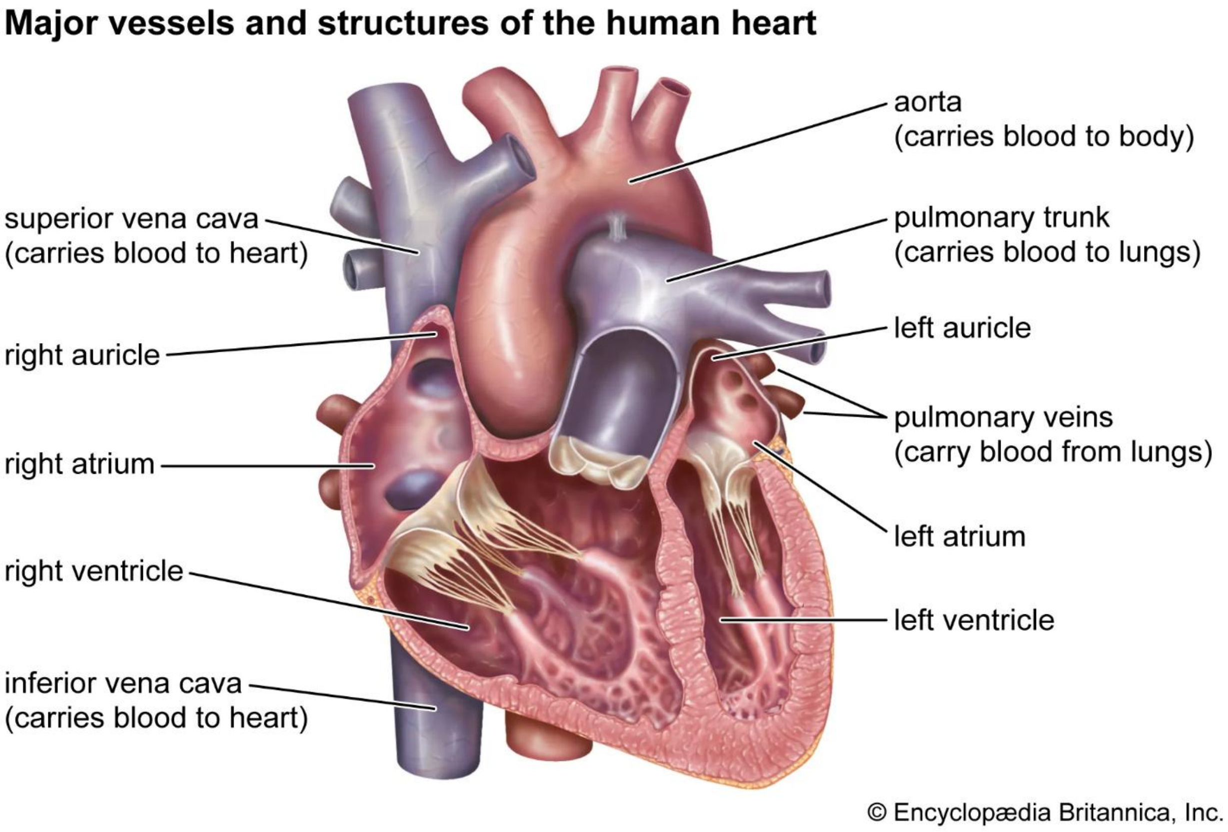

Fig 1.1 Longitudinal section of the Human Heart

Reproduced from “Heart,” by Britannica (n.d.), retrieved from

https://www.britannica.com/science/heart.

1.1.4 CLINICAL ASSESSMENT OF THE HEART.

An essential part of cardiovascular evaluation and a key factor in the diagnosis of cardiac disorders is the clinical examination of the heart. It uses a methodical process that include auscultation, percussion, palpation, and inspection. Even though cardiology has changed due to technical advancements like echocardiography and electrocardiograms, a comprehensive physical examination is still essential, particularly for initial patient evaluation and in situations with limited diagnostic resources.

Usually, the examination starts with an inspection, in which the doctor looks for any obvious pulsations, scars, abnormalities, or indications of heart failure, including cyanosis or jugular vein distention, on the anterior chest wall. Patients with persistent heart enlargement may exhibit precordial bulges. Important hints regarding underlying heart pathology can also be found by observing the patient's respiratory effort, skin tone, and any outward manifestations such clubbing, edema, or xanthomata (Epstein and Perloff, 2011).

The next step is palpation, in which the examiner feels for the apex beat, which is often found near the midclavicular line in the fifth intercostal area. The size and function of the left ventricle can be inferred from the position, size, and nature of the apex beat. A dispersed or misplaced apex beat could be a sign of dilatation or hypertrophy of the left ventricle. Additionally, the examiner may palpate for parasternal heaves that could indicate right ventricular hypertrophy and thrills, which are perceptible murmurs (Bickley and Szilagyi, 2017).

Although it is not as frequently utilized in contemporary practice, percussion can be useful in estimating the cardiac boundaries, especially when imaging is not available. In order to identify the change from resonant lung fields to the heart's dullness, the chest wall is tapped. Percussion can provide initial information in situations when more sophisticated instruments are not available, despite its low sensitivity and specificity (Talley and O'Connor, 2018).

The most important and instructive aspect of the cardiac examination is auscultation. A stethoscope is used to listen for murmurs, heart sounds, and other sounds like clicks or rubs. The aortic, pulmonic, tricuspid, and mitral valve sections are the four main locations where the clinician methodically listens. Timing, splitting, and strength of the first and second heart sounds (S1 and S2) are assessed. Further diagnostic information can be obtained from other sounds, such as the fourth heart sound (S4), which is linked to stiff ventricles, or the third heart sound (S3), which may suggest cardiac failure (Levine and Harvey, 2019). Timing (systolic or diastolic), pitch, location, radiation, and reaction to body posture changes or Valsalva exercises are all factors used to evaluate murmurs.

Beyond, the cardiovascular assessment is supported by the peripheral examination of the chest. Systemic involvement can be detected by looking for symptoms such peripheral edema, delayed capillary refill, and irregular pulse character. For instance, a narrow pulse pressure may signify cardiogenic shock, but a collapsing (water hammer) pulse may indicate aortic regurgitation (Kumar and Clark, 2020).

Crucially, the doctor needs to include the results from every aspect of the evaluation. For example, a systolic murmur extending to the carotids, a misplaced apex beat, and the absence of a second heart sound may all indicate significant aortic stenosis. Clinical expertise, a solid grasp of heart physiology, and thorough linkage with patient history are necessary for this kind of synthesis of physical signals (Macleod *et al*., 2013).

1.1.5 TYPICAL CARDIOVASCULAR CONDITIONS.

Cardiovascular diseases remain the leading cause of mortality worldwide, encompassing a broad range of disorders that affect the heart and blood vessels. These include hypertension, coronary artery disease, arrhythmias, heart failure, and congenital cardiac malformations. Given their global burden and profound health consequences, comprehensive understanding of these conditions is essential for effective prevention, diagnosis, and management. Hypertension—commonly labelled the “silent killer”—is the most widespread cardiovascular disorder and is defined by a chronic elevation in arterial blood pressure. Because it is usually asymptomatic, it progressively damages major organs such as the heart, kidneys, and blood vessels. According to Mancia and Grassi (2014), sustained high blood pressure places continuous strain on the cardiovascular system, substantially increasing the risk of stroke, renal failure, and heart failure. Risk factors include age, hereditary predisposition, obesity, poor dietary patterns, and alcohol consumption. If left uncontrolled, hypertension may result in vascular injury and left ventricular hypertrophy (Bonow et al., 2012).

Coronary artery disease (CAD) arises from the formation of atherosclerotic plaques within the coronary vessels, thereby restricting the heart’s blood supply. Clinical manifestations range from angina pectoris to myocardial infarction. The disease begins with endothelial dysfunction, followed by inflammation and lipid accumulation. Plaque rupture and subsequent thrombosis are key events leading to acute coronary syndromes (Fuster et al., 2017). Major contributors to its progression include smoking, diabetes, hyperlipidaemia, and physical inactivity (Zipes et al., 2018). Heart failure is defined as the inability of the heart to maintain adequate cardiac output, leading to symptoms such as dyspnoea, fatigue, and fluid retention. It is classified into heart failure with reduced ejection fraction (HFrEF) and preserved ejection fraction (HFpEF). Common underlying causes include long-standing hypertension, cardiomyopathy, and myocardial infarction. The condition progresses through mechanisms such as ventricular remodelling, diminished cardiac output, and neurohormonal activation (Yancy et al., 2013). Therapeutic strategies typically involve diuretics, ACE inhibitors, and beta-blockers. Cardiac arrhythmias are disturbances in the heart’s electrical conduction pathways and may range from benign premature beats to severe, life-threatening rhythms such as ventricular tachycardia or fibrillation. Atrial fibrillation (AF) is the most common arrhythmia and significantly increases the risk of stroke due to thrombus formation in the atria. January et al. (2014) identify hypertension, valvular disorders, and advancing age as major risk factors. Diagnosis is confirmed through electrocardiography, while management includes rate control, anticoagulation therapy, and catheter ablation. Congenital heart defects (CHDs) are structural abnormalities present at birth and may involve septal defects, malformed valves, or complex malformations such as tetralogy of Fallot. Advances in prenatal diagnosis and surgical interventions have markedly improved prognosis. Hoffman and Kaplan (2002) report an incidence of approximately 8 cases per 1000 live births. Clinical presentation varies from asymptomatic forms to severe manifestations such as cyanosis, feeding difficulties, or early heart failure. Management depends on the specific defect and may involve observation, pharmacologic therapy, or surgical correction.

1.1.6 PREVALENCE OF CARDIOVASCULAR DISEASES.

With an estimated 17.9 million deaths year, or almost 32% of all deaths worldwide, cardiovascular disease (CVD) continues to be the major cause of mortality worldwide (World Health Organization, 2021). More than 75% of fatalities from CVD occur in low- and middle-income countries, where access to early identification and treatment is frequently restricted. This means that the burden is not just confined to high-income countries. Hypertensive heart disease, stroke, and coronary artery disease are the most common types. Lifestyle factors like smoking, excessive alcohol consumption, poor food, and physical inactivity have led to an increase in prevalence in many areas, especially among younger populations (Roth *et al*., 2020). This expanding tendency poses a serious public health risk that calls for focused management and prevention measures.

1.1.7 SYMPTOMS OF CARDIOVASCULAR DISEASES.

Although the symptoms of cardiovascular disease (CVD) differ from one condition to another, they frequently overlap because of common underlying processes such as structural abnormalities or decreased blood flow. Angina, or chest pain or discomfort, palpitations, exhaustion, lightheadedness, and lower limb edema from fluid retention are typical symptoms. Patients may have nausea, perspiration, and crushing chest pain that radiates to the left arm or jaw in situations such myocardial infarction (Bonow *et al*., 2012). Peripheral edema, orthopnea, and exertional dyspnea are common symptoms of heart failure (Yancy *et al*., 2013). Regular screening is essential for early detection because some types of CVD, like hypertension or early atherosclerosis, may not show any symptoms for years (Kumar and Clark, 2020).

1.1.8 CHANGES IN LIFESTYLE FOR THE TREATMENT OF HEART DISEASE.

A key component of managing and preventing cardiovascular disease is changing one's lifestyle. Adopting a heart-healthy diet, getting more exercise, keeping a healthy weight, stopping smoking, consuming alcohol in moderation, and managing stress are all important elements. These modifications aid in the management of risk factors like obesity, diabetes, hypertension, and hyperlipidemia. For instance, regular aerobic exercise improves lipid profiles, lowers blood pressure, and improves endothelial function. Improved metabolic outcomes and fewer cardiovascular events are linked to dietary interventions like the DASH or Mediterranean diets. Quitting smoking dramatically reduces the risk of stroke and coronary heart disease. When regularly implemented, these lifestyle changes improve outcomes for individuals with pre-existing conditions as well as prevent cardiovascular events (Ornish *et al*., 1998; Estruch *et al*., 2013).

1.1.9 THE USE OF PHARMACEUTICALS TO TREAT CARDIOVASCULAR DISEASE

In the treatment of cardiovascular disease, pharmacological therapy is still essential since it aims to lessen symptoms, avoid complications, and increase long-term survival. In order to control high blood pressure and lower the risk of stroke and heart failure, antihypertensive medications such ACE inhibitors, angiotensin receptor blockers (ARBs), calcium channel blockers, and thiazide diuretics are frequently used. The cornerstone of cholesterol management, statins considerably lower the risk of atherosclerotic cardiovascular events. Furthermore, high-risk individuals who continue to have hyperlipidemia in spite of statin therapy are being treated with innovative drugs like PCSK9 inhibitors (Mach *et al*., 2019). Due to better mortality and symptom control, sacubitril/valsartan has frequently taken the position of conventional ACE inhibitors in the treatment of heart failure (McDonagh *et al*., 2021). Antiplatelet medications, such as aspirin and P2Y12 inhibitors, are essential for patients with coronary stents and for the secondary prevention of myocardial infarction. Therapy selection is becoming more and more customized based on risk stratification algorithms, genetic variables, and patient comorbidities.

1.1.10 SURGICAL TECHNIQUES FOR THE TREATMENT OF HEART CONDITIONS.

In the treatment of severe cardiovascular disease, surgery is still essential, especially when medication and lifestyle changes are not enough. Patients with severe coronary artery disease, particularly those with diabetes or multivessel disease, may benefit from coronary artery bypass grafting (CABG), a frequent surgical treatment intended to restore blood flow. It has been demonstrated to lower the risk of myocardial infarction and increase survival (Neumann *et al*., 2019). Another important option is valve replacement or repair surgery, especially for patients with congenital valve abnormalities, mitral regurgitation, or aortic stenosis. Because of its minimally invasive nature and positive results, transcatheter aortic valve implantation (TAVI) has grown in popularity among high-risk and elderly patients (Vahanian *et al*., 2022). In order to manage arrhythmias and prevent sudden cardiac death, procedures including pacemaker implantation, cardiac resynchronization therapy (CRT), and implanted cardioverter-defibrillators (ICDs) are essential. These surgical methods have greatly improved survival and quality of life for individuals with severe cardiovascular problems and are frequently customized to the patient's clinical situation.

1.1.11 CONTINUOUS MONITORING FOR THE MANAGEMENT OF CARDIOVASCULAR DISEASE**.**

In order to ensure that treatment objectives are fulfilled, problems are identified early, and medication is modified as necessary, monitoring and evaluation are crucial to the long-term management of cardiovascular disease. Regular measurements of blood pressure, heart rate, lipid profile, blood glucose levels, kidney function, and weight are all part of continuous assessment. Tools including electrocardiograms (ECG), Holter monitoring, echocardiogram, and implanted devices like pacemakers and defibrillators offer vital diagnostic and prognostic data for patients with heart failure or arrhythmias (McDonagh *et al*., 2021).

In order to improve patient involvement and lower hospital readmission rates, wearable technology and remote monitoring technologies are being utilized more and more to measure cardiac rhythm and identify irregularities in real time (Kitsiou *et al*., 2022). Troponins and NT-proBNP are two other biomarkers that help assess the course of the disease and direct treatment. In order to lower morbidity and enhance quality of life for patients with cardiovascular disorders, regular follow-up visits enable medical professionals to evaluate medication adherence, reinforce lifestyle modifications, and implement prompt interventions.

1.2.0 MEDICINAL PLANTS

A General Introduction

For millennia, medicinal plants have been the main source of healthcare in many societies, and they still have a big impact on modern medicine. Alkaloids, flavonoids, tannins, saponins, and terpenoids are among the naturally occurring bioactive substances found in these plants. These substances have anti-inflammatory, antioxidant, antibacterial, and cardioprotective actions. They are particularly widely used in underdeveloped nations, where they frequently act as easily accessible and reasonably priced substitutes for synthetic medications. According to estimates from the World Health Organization, almost 80% of people worldwide get their basic medical care from traditional medicine, which is mostly made up of plant-based treatments (WHO, 2019). In addition to being essential to conventional medical systems like Ayurveda, TCM, and African ethnomedicine, medicinal plants have also aided in the advancement of contemporary pharmacology. Aspirin (derived from willow bark), digoxin (derived from Digitalis purpurea), and quinine (derived from Cinchona species) are only a few of the well-known pharmaceutical medications that have botanical origins (Newman and Cragg, 2020).

Because medicinal plants may provide safer, more natural treatments with fewer side effects, scientific interest in them has increased significantly. Numerous species are being investigated by researchers to cure chronic illnesses like diabetes, cancer, heart disease, and neurological disorders. Azadirachta indica, Moringa oleifera, and *Alstonia boonei* for instance, have demonstrated encouraging pharmacological qualities in the regulation of metabolism and the cardiovascular system. Nevertheless, issues with standardization, quality assurance, dosage control, and possible herb-drug interactions still exist despite their advantages. Strict scientific verification, legal frameworks, and incorporation into evidence-based healthcare systems are necessary to guarantee the security and effectiveness of medicinal plants.

1.2.1 AN OVERVIEW OF THE HISTORY AND SIGNIFICANCE OF MEDICINAL PLANTS.

The usage of medicinal plants has been around since ancient times, and they served as the basis for indigenous African practices, traditional Chinese medicine, and Indian Ayurvedic systems. Hundreds of herbal treatments for a variety of illnesses are documented in ancient manuscripts such as the Chinese "Shennong Ben Cao Jing" and the Egyptian Ebers Papyrus (c. 1550 BCE). These early systems, which were derived from empirical observations and transmitted through the generations, ultimately impacted the advancement of contemporary medicine and pharmacology (Fabricant and Farnsworth, 2001).

Medicinal plants are important because of their long-standing impact on healthcare around the world. Plant-based medicines continue to be used as a primary or supplemental treatment by a significant section of the global population. Many pharmaceutical medications, such as morphine, quinine, and artemisinin, have been derived from their diverse array of bioactive chemicals. Apart from their therapeutic potential, medicinal plants are frequently accessible and culturally meaningful, especially in environments with low resources where synthetic medications may be expensive or unavailable (World Health Organization, 2019). The need for scientific confirmation and the preservation of medicinal plant resources has been further underscored by the growing interest in natural and holistic health around the world.

1..2.2 AN OVERVIEW OF TRADITIONAL CHINESE MEDICINE (TCM).

With a history spanning more than 2,500 years, Traditional Chinese Medicine (TCM) is among the world's oldest and most extensive therapeutic systems. The foundation of TCM, which has its roots in ancient Chinese philosophy, is the idea of harmony and balance both within the body and between the individual and their surroundings. Its fundamental theories, which direct disease diagnosis, treatment, and prevention, include the ideas of Qi (vital energy), Yin and Yang, and the Five Elements (Zhou *et al*., 2016). Herbal medicine, acupuncture, moxibustion, cupping treatment, massage (Tui Na), and nutritional therapy are just a few of the many disciplines that fall under the umbrella of Traditional Chinese Medicine (TCM). Thousands of plant, animal, and mineral compounds are used in herbal therapy, which is especially important for reestablishing physiological equilibrium. Herbal formulation and TCM philosophy have been based on classic books such as the Shennong Ben Cao Jing and Huangdi Neijing (Yellow Emperor's Inner Canon). TCM prioritizes long-term health maintenance and the restoration of the body's self-regulatory systems over treating disease symptoms alone (Tang *et al*., 2018). TCM's holistic approach, which incorporates the mental, emotional, and spiritual facets of health, is what makes it significant. It is extensively utilized in Asia and is becoming more commonly acknowledged worldwide as a supplemental treatment for long-term ailments like gastrointestinal issues, arthritis, cardiovascular diseases, and illnesses linked to stress. The effectiveness of numerous TCM treatments has been confirmed by scientific studies. For example, acupuncture has been shown to be useful in managing pain and lowering blood pressure, and several herbal components have demonstrated cardioprotective, antioxidant, and anti-inflammatory qualities (Wang *et al*., 2020).

Even with its extensive use, TCM still has issues with integration with contemporary biomedical systems, quality control, and standardization. However, there are now more opportunities for TCM to be incorporated into evidence-based therapy due to the growing interest in individualized and holistic healthcare. TCM is employed in hospitals alongside mainstream medicine in China and other parts of the world to give patients more treatment alternatives.

1.2.3. AN OVERVIEW OF INDIAN TRADITIONAL MEDICINE.

Indian traditional medicine particularly Ayurveda represents one of the world's oldest holistic medical frameworks, with origins extending back more than 3,000 years. The term Ayurveda is derived from the Sanskrit words *Ayur* (life) and *Veda* (knowledge), collectively meaning "the science of life." This system emphasizes the interconnectedness of the body, mind, and spirit, aiming to maintain health, prevent disease, and manage illness through natural means (Patwardhan et al., 2015).

Ayurveda is built upon the concept of three fundamental energies, known as doshas: Pitta (fire and water), Kapha (earth and water), and Vata (air and space). Each individual is believed to possess a unique combination of these doshas, and disease arises when this balance is disrupted. Ayurvedic diagnosis typically involves evaluating physical, psychological, and environmental factors, often incorporating methods such as pulse analysis (Nadi Pariksha), tongue examination, and lifestyle assessment. Therapeutic approaches include herbal formulations, dietary modifications, yoga, meditation, massage therapies such as Abhyanga, and detoxification procedures like Panchakarma (Tillu et al., 2019). Herbal medicine forms a central component of the Ayurvedic tradition. Plants such as ashwagandha (*Withania somnifera*), turmeric (*Curcuma longa*), neem (*Azadirachta indica*), and tulsi (*Ocimum sanctum*) are widely used for their antioxidant, anti-inflammatory, and immunomodulatory effects. These herbal agents are frequently combined to enhance therapeutic synergy and minimize potential toxicity (Ekor, 2014). The significance of Indian traditional medicine lies in its preventive approach, holistic philosophy, and cultural relevance. It remains extensively practiced across India, and the Indian government formally supports it through the Ministry of AYUSH (Ayurveda, Yoga & Naturopathy, Unani, Siddha, and Homoeopathy). Globally, Ayurvedic medicine is attracting increasing interest due to its natural orientation and compatibility with integrative health models. Nevertheless, challenges remain—particularly with respect to standardization, scientific validation, and regulatory oversight—which must be addressed to ensure safety, efficacy, and broader international acceptance (Patwardhan and Chandran, 2017).

1.2.4 AN OVERVIEW OF AFRICAN TRADITIONAL MEDICINE (ATM)

Indigenous health knowledge, beliefs, and practices utilized for the diagnosis, prevention, and treatment of physical and mental disorders throughout Africa are collectively referred to as African Traditional Medicine (ATM). ATM is a holistic system that incorporates herbal treatments, spiritual ceremonies, and community-based care, and it has deep roots in culture, spirituality, and nature. For a sizable section of the African population, particularly in rural and underserved areas, it has been a vital source of healthcare for generations (World Health Organization, 2013). The use of medicinal herbs, many of which have demonstrated pharmacological qualities, is a fundamental component of ATM. Commonly used to cure ailments ranging from infections and pain to diabetes and hypertension are plants including Zanthoxylum zanthoxyloides, Vernonia amygdalina, Moringa oleifera, and *Alstonia boonei*. Depending on the area, traditional healers, known as Babalawo, Sangoma, or Dibia, often give treatments. Respected members of their communities, these practitioners are thought to have both spiritual and scientific knowledge, which includes the capacity to execute protective or purifying rites, call upon ancestor guidance, and interpret dreams (Abdullahi, 2011). ATM is noteworthy for its contributions to contemporary pharmacology in addition to its accessibility and cultural significance. African medicinal plants are the source of several pharmaceutical medications, including quinine and artemisinin. Furthermore, research and preservation of ATM provide important information on sustainable healthcare, biodiversity, and culturally competent medical procedures (Willcox and Bodeker, 2020). Despite being widely used, ATMs still have issues with standardization, safety, and interaction with official healthcare systems. There are continuous initiatives to safeguard indigenous knowledge, regulate traditional medicine, and advance scientific validation. ATM has been included into national health policy in a number of African nations, and collaborations between traditional and contemporary medical professionals are being promoted in an effort to enhance public health outcomes (WHO, 2013).

1.2.5 MEDICINAL PLANTS IN THE WEST AFRICA CULTURE.

West African culture is heavily reliant on medicinal plants, which serve as the foundation of traditional medical systems and provide substantial contributions to both healthcare and cultural identity. Traditional medicine is firmly anchored in local knowledge, spirituality, and social customs throughout the region, which includes nations like Nigeria, Ghana, Senegal, Mali, and Burkina Faso. In many cultures, healing is a social and spiritual process in addition to a physical one. According to Abdullahi (2011), traditional healers—also referred to as Babalawo in Nigeria, Marabouts in Senegal, and Wogons in Mali, are seen as essential stewards of ancestral wisdom and frequently learn their craft through apprenticeship and oral tradition. Throughout West Africa, a vast array of native medicinal plants are utilized to cure conditions like infections, diabetes, hypertension, and malaria. Common species include Vernonia amygdalina (bitter leaf, used for diabetes and malaria), *Alstonia boonei* (used for fever, hypertension, and pain), Azadirachta indica (neem, used for inflammation and skin illnesses), and Zanthoxylum zanthoxyloides (used for toothaches and digestive issues). Depending on the ailment being treated, these herbs are usually prepared as decoctions, infusions, or powders and can be applied topically, taken orally, or inhaled (Agyare *et al*., 2016). A sizable section of the populace in nations like Ghana and Nigeria still receives treatment from traditional medicine, especially in rural areas with little access to modern healthcare. In addition to their therapeutic applications, these plants are deeply ingrained in the people's spiritual and cultural lives. A worldview that views health as harmony between the body, spirit, community, and environment can be reflected in healing ceremonies, which may include rituals, chants, libations, and offerings (Ategbo *et al*., 2022).

West Africa's rich ethnobotanical expertise has gained more attention as a result of the growing interest in natural medicines around the world. Numerous pharmacological characteristics of West African medicinal herbs, such as their antibacterial, antihypertensive, antioxidant, and antimalarial actions, have been validated by scientific investigation. However, there are still issues with sustainable use, scientific validation, and dosage uniformity. under order to preserve biodiversity, document this knowledge, and incorporate safe traditional methods into larger healthcare systems, national and regional initiatives are currently under progress (Abbiw, 2020).

1.2.6 EAST AFRICAN CULTURE AND MEDICINAL PLANTS.

Medicinal plants are essential to traditional healing methods in East African culture because they offer therapeutic benefits as well as a link to ancestry. Plant-based medicines have long been used to treat a variety of illnesses in nations including Kenya, Tanzania, Uganda, Ethiopia, and Rwanda. These customs have their origins in indigenous knowledge systems, known as balew in Ethiopia and waganga in Swahili-speaking areas, which have been down through the generations by traditional healers (Okello & Ssegawa, 2007). According to the holistic perspective of East African traditional medicine, illness arises from imbalances between the individual, communal, and spiritual forces in addition to those within the body. As a result, spiritual rites and herbal remedies are both used in healing. In the area, Prunus africana is used to treat prostate issues, Ocimum suave is used to treat cough and fever, Warburgia ugandensis is used to treat respiratory infections and malaria, and Aloe secundiflora is used to treat wounds, stomach ulcers, and skin conditions. Depending on the condition and cultural custom, remedies are frequently made as decoctions, infusions, powders, or topical treatments (Kokwaro, 2009).

Traditional medicine is still the mainstay of therapy in rural East Africa, where access to contemporary healthcare is scarce. Many people use herbal treatment in addition to conventional care, even in urban areas. Although growing urbanization and deforestation are obstacles to sustainability and availability, cultural reverence for nature and biodiversity aids in the conservation of medicinal plants (Yineger *et al.*, 2008).

1.2.7 THE CONTINUED IMPORTANCE OF MEDICINAL PLANTS IN THE HEAALTHCARE SYSTEM

Medicinal plants are still very important in today's worldwide healthcare systems, even with the development of contemporary medications and biotechnology. Their continued use stems from their practicality, cost, and demonstrated medicinal efficacy in addition to cultural traditions. A sizable section of the populace still uses plant-based medicines as their main source of healthcare in various regions of Africa, Asia, and Latin America. For their fundamental medical needs, up to 80% of people in underdeveloped nations rely on traditional medicine, which is mostly based on medicinal plants, according to the World Health Organization (WHO, 2019). Medicinal plants are still essential to the development of new drugs today. Many pharmacological medications are either directly derived from plants or are based on phytochemical components that are present in conventional medicine. Examples include digoxin from Digitalis purpurea for heart failure, morphine from Papaver somniferum for pain management, and artemisinin from Artemisia annua for malaria. Similar to this, *Alstonia boonei* , which is commonly used in West Africa, has demonstrated strong hypotensive, antipyretic, and anti-inflammatory properties. It has historically been used to cure fever, rheumatism, malaria, and hypertension. Its bioactive ingredients, including flavonoids and alkaloids, have drawn interest from researchers (Akinmoladun *et al*., 2020).

Additionally, integrative and alternative medicine systems are increasingly embracing medicinal plants. Plant-based treatments are used to maintain the immune system, manage chronic diseases, and promote general wellness in both high- and low-income nations. Their perceived safety, accessibility, and comprehensive qualities are frequently what make them appealing (Ekor, 2014).

1.2.8 THE VALUE OF MEDICINAL PLANTS IN PHARMACOLOGICAL RESEARCH.

The various pharmacological actions of medicinal plants are well known. Alkaloids, flavonoids, tannins, terpenoids, and saponins are some of their naturally occurring bioactive components that have shown promise in treating a variety of illnesses. These pharmacological characteristics not only support conventional applications but also offer guidance for the creation of novel medications. Here are five noteworthy instances of each of the most important pharmacological characteristics of therapeutic plants.

(a.) Activity of Antioxidants

Antioxidants counteract free radicals and stop oxidative stress, which is linked to aging and long-term conditions like diabetes, heart disease, and cancer.

Examples:

. Quercetin and chlorogenic acid are found in Moringa oleifera (Siddhuraju and Becker, 2003). • Turmeric, or Curcuma longa, is high in curcumin (Aggarwal and Harikumar, 2009).

- Green tea, or Camellia sinensis, has a high catechin content (Cabrera *et al*., 2006).

- Flavonoids and sesquiterpene lactones are found in Vernonia amygdalina (Erasto *et al*., 2007).

Eugenol and phenolic chemicals are found in Ocimum gratissimum (Prakash and Gupta, 2005).

(b.) Properties that Reduce Inflammation

Many chronic disorders are caused by inflammation. Asthma, inflammatory bowel disorders, and arthritis can all benefit from medicinal plants that have anti-inflammatory properties.

Examples:

- Altonia boonei: Used to treat inflammation and fever in West Africa (Akinmoladun *et al*., 2020)

. Gingerol is found in Zingiber officinale, or ginger (Grzanna *et al*., 2005).

. Boswellic acids are produced by Boswellia serrata (Ammon, 2006).

- Ashwagandha, or Withania somnifera, contains withanolides (Mishra *et al*., 2000).

- White willow, or Salix alba, is the source of salicin, which is an aspirin precursor (Mahdi *et al*., 2006).

(c.) Antimicrobial Action

The use of medicinal plants in infectious disorders is supported by the fact that many of them have antibacterial properties against bacteria, fungus, and viruses.

Examples:

- Neem, or Azadirachta indica, has antifungal and antibacterial properties (Subapriya and Nagini, 2005).
- Allicin is found in garlic, or Allium sativum (Ankri and Mirelman, 1999).

According to Eliaissi *et al*. (2012), eucalyptus globulus is used to treat respiratory infections.

- Thyme, or Thymus vulgaris, is high in thymol (Rota *et al*., 2008).

According to Arima and Danno (2002), Psidium guajava, or guava, is effective against gastrointestinal infections.

(d.) Anticancer Characteristics

Numerous substances produced from plants have been demonstrated to stop the growth of cancer cells, trigger apoptosis, and stop them from spreading.

Examples:

- Vincristine and vinblastine are derived from Catharanthus roseus (Noble, 1990).
- Paclitaxel (Taxol) is derived from Taxus brevifolia (Wani *et al*., 1971).
- Curcuma longa: Curcumin has anticancer properties (Aggarwal and Harikumar, 2009),
- Camptotheca acuminata is the source of camptothecin (Wall and Wani, 1966),.
- Annonaceous acetogenins are found in Annona muricata, often known as sorsop (Oberlies *et al*., 1999).

(e.) Antidiabetic Effects

By increasing insulin secretion or improving glucose metabolism, plants having hypoglycemic properties can help manage type 2 diabetes.

Examples:

- Bitter melon, or Mordica charantia, improves insulin sensitivity (Joseph and Jini, 2013).

Gymnema sylvestre is well-known for its ability to reduce blood sugar levels (Shanmugasundaram *et al*., 1990).

- Fenugreek, or Trigonella foenum-graecum, lowers blood sugar (Ribes *et al*., 1986).
- Holy basil, or Ocimum sanctum, increases glucose tolerance (Rai *et al*., 1997).

- According to Akah and Okafor (1992), vernonia amygdalina lowers blood glucose levels.

1.2.9 THE ECONOMIC SIGNIFICANCE OF MEDICINAL PLANTS

Medicinal plants are valued not only for their therapeutic properties but also for their significant economic importance at both local and global levels. As the demand continues to rise for natural health products, herbal remedies, and phytopharmaceuticals, these plants have become essential raw materials for industries such as pharmaceuticals, cosmetics, food, and wellness. Their cultivation, processing, and commercialization provide income for millions of people, particularly in developing regions where traditional medicine remains integral to healthcare and cultural identity. For many rural communities across Africa, Asia, and Latin America, the harvesting and sale of medicinal plants serve as a major source of livelihood. In countries such as Nigeria and India, the medicinal plant trade supports thousands of households involved in cultivation, local market distribution, and processing. In Nigeria, species such as *Alstonia boonei*, *Vernonia amygdalina*, and *Zingiber officinale* play a prominent role in the herbal medicine sector, which contributes billions of naira annually to the national economy (Akinmoladun et al., 2020). The global market for medicinal plants is also expanding rapidly. According to the World Health Organization, the value of the herbal medicine industry was projected to surpass USD 111 billion by 2023, driven by increasing consumer preference for plant-based health products (WHO, 2019). Highly traded medicinal species include *Withania somnifera*, *Curcuma longa*, and *Panax ginseng*, with China and India among the leading exporters. Major importers include the United States and European countries, where botanical supplements and herbal therapeutics continue to gain popularity.

1.2.10 Application of Medicinal Plants in the Management of Cardiovascular Diseases

Given that cardiovascular diseases (CVDs) remain the foremost cause of morbidity and mortality worldwide, the search for affordable and effective treatment options persists. Medicinal plants have historically played a central role in cardiovascular therapy within various traditional medical systems, including Ayurveda, Traditional Chinese Medicine (TCM), and African Traditional Medicine. Contemporary scientific research has validated the pharmacological potential of many of these plant-based remedies, demonstrating their antihypertensive, lipid-lowering, antioxidant, anti-inflammatory, and antiplatelet activities, all of which contribute to improved cardiovascular function.

(a.) *Alstonia Boonei*

Alstonia boonei is indigenous to West Africa and is used extensively in ethnomedicine to treat inflammatory conditions, fever, and hypertension. Its hypotensive and anti-inflammatory qualities are thought to be attributed to the presence of alkaloids, tannins, flavonoids, and saponins, as shown by phytochemical studies. In animal models, studies have demonstrated that extracts from A. boonei lower systolic and diastolic blood pressure, potentially through the promotion of diuresis and vasodilation (Akinmoladun *et al*., 2020; Odugbemi, 2008). Ajaiyeoba *et al*. (2003) state that *Alstonia boonei* has also shown antioxidant activity, which is essential in reducing oxidative damage to vascular tissues, a major mechanism in the development of atherosclerosis.

(b.) Garlic, or Allium sativum

One of the most researched medicinal herbs with cardioprotective qualities is garlic. For millennia, people from many cultures have used it to treat circulatory diseases, high blood pressure, and excessive cholesterol.

Garlic's cardiovascular advantages are largely due to its active ingredient, allicin. It lowers total and LDL cholesterol, inhibits platelet aggregation, and increases nitric oxide generation to promote vasodilation, all of which can reduce the risk of thrombotic events (Ried *et al*., 2013; Banerjee and Maulik, 2002). Garlic supplements can result in moderate but significant drops in blood pressure, especially in people with hypertension, according to meta-analyses (Ried *et al*., 2008). Additionally, endothelial dysfunction, a crucial stage in the development of CVDs, is prevented by its antioxidant qualities (Rahman and Lowe, 2006).

(c.) Hawthorn, or Crataegus monogyna

Hawthorn has been used for centuries in European herbal medicine, particularly for managing arrhythmias and congestive heart failure. Its therapeutic effects are largely attributed to the high levels of flavonoids and oligomeric procyanidins present in its leaves, flowers, and berries. Pharmacological studies indicate that *Crataegus* species enhance coronary circulation, improve myocardial oxygen efficiency, and exert mild positive inotropic activity, making hawthorn extracts especially beneficial in early-stage heart failure (Tassell et al., 2010; Pittler et al., 2008). Clinical investigations further demonstrate that hawthorn supplementation improves exercise capacity and reduces heart failure symptoms such as fatigue and shortness of breath (Walker et al., 2002).

(d.) Hibiscus sabdariffa (Roselle)

Hibiscus sabdariffa, widely consumed in Africa, the Caribbean, and parts of Asia, is traditionally prepared as tea using its deep-red calyces. The plant's pharmacological activity is linked to its rich content of anthocyanins, flavonoids, and organic acids, which provide strong antioxidant and antihypertensive effects. Several clinical trials have shown that hibiscus tea significantly reduces systolic and diastolic blood pressure in individuals with prehypertension and mild hypertension (McKay et al., 2010; Herrera-Arellano et al., 2007). Its antihypertensive mechanisms involve diuretic effects, endothelial-dependent vasodilation, and inhibition of angiotensin-converting enzyme (ACE). In addition, hibiscus extracts have been reported to improve lipid profiles by increasing HDL cholesterol while reducing LDL cholesterol and triglycerides, thereby lowering overall cardiovascular risk (Ajay et al., 2007).

(e.) Terminalia arjuna

Terminalia arjuna is a well-established cardiotonic herb in Ayurvedic medicine, with its bark traditionally used to treat angina, hypertension, and heart failure. The plant contains several bioactive constituents, including flavonoids, tannins, glycosides, and saponins. Research findings indicate that *T. arjuna* enhances left ventricular function, decreases blood pressure, and alleviates ischemic symptoms (Dwivedi and Jauhari, 1997). Clinical studies further show that, when used alongside conventional therapy, it improves myocardial oxygen consumption and reduces the frequency of angina attacks (Dwivedi and Gupta, 2002). Its antioxidant and anti-inflammatory activities also contribute to maintaining arterial health and protecting the endothelium, which are crucial for preventing atherosclerosis and its complications (Jagtap and Bhutani, 2005).

1.2.11 OBSTACLES IN THE STUDY AND APPLICATION OF MEDICINAL PLANTS.

A number of obstacles prevent medicinal plants from being effectively researched, validated, and widely incorporated into contemporary healthcare systems, despite their enormous therapeutic potential and economic worth.

(a.) Inadequate Standardization and Scientific Validation

The lack of adequate scientific proof of medicinal plants' safety, effectiveness, and dose is a significant barrier to their use. Few traditional treatments have undergone thorough clinical studies, despite the fact that many are founded on decades of empirical experience. Standardization is challenging because of the heterogeneity in phytochemical composition brought about by variations in plant species, growth environments, and preparation techniques (Ekor, 2014). It is difficult to guarantee consistency in therapeutic results in the absence of standardized formulations.

(b.) Adulteration and Quality Control

Inappropriate processing, adulteration, or contamination commonly degrade the quality and purity of herbal products. Particularly when gathered from contaminated areas or handled in unsanitary conditions, herbal products may include harmful metals, pesticide residues, or microbiological pollutants (Kunle *et al.*, 2012). Safety risks are further raised by adulteration using synthetic medications or non-declared chemicals, especially in unregulated marketplaces.

(c.) Loss of Biodiversity and Overharvesting

Many significant species have been lost as a result of an over-reliance on therapeutic herbs collected from the wild. Unsustainable harvesting methods endanger the ecological balance of habitats and plant biodiversity. Due to inadequate conservation efforts and strong commercial demand, species like Prunus africana and Taxus brevifolia are currently endangered (Hamilton, 2004). Concerns about long-term sustainability are exacerbated by the way that climate change impacts the distribution and effectiveness of medicinal plants.

(d.) Intellectual Property and Sharing of Benefits

The application of indigenous knowledge in drug discovery has brought up moral and legal concerns about benefit-sharing and intellectual property rights. When their expertise results in commercial items, traditional knowledge holders—who are frequently from rural or Indigenous communities—may not be acknowledged or compensated (Scholz *et al*., 2004). Cooperation between communities and researchers may be discouraged by the absence of legal mechanisms for fair benefit-sharing.

(e.) Inadequate Regulations

Herbal medicine is a gray area under many countries' regulations. Many herbal products are promoted without strict regulatory approval or post-marketing supervision, in contrast to pharmaceutical medications. Labeling, safety claims, and therapeutic indications become inconsistent as a result. Patient safety is jeopardized and public trust is eroded in the absence of robust national regulations and enforcement mechanisms (WHO, 2019).

1.3.0 AN OVERVIEW OF *ALSTONIA BOONEI*.

The tall, deciduous *Alstonia boonei*, De Wild tree is a member of the Apocynaceae family and is indigenous to tropical West Africa. Often called "cheese wood" or "God's tree," it is utilized extensively in African traditional medicine. With its whorled leaves, gray bark, and latex-filled stem, the tree can reach a height of 45 meters. In Nigeria, Ghana, Sierra Leone, and Cameroon, it grows well in rainforest and damp savannah areas (Burkill, 1985).

Traditionally, fever, pain, malaria, rheumatism, and high blood pressure have all been treated using various portions of the plant, especially the stem bark, root, and leaves. The bark's wide range of therapeutic applications is demonstrated by the fact that decoctions of it are used to treat inflammatory and hypertensive diseases in various communities (Akinmoladun *et al*., 2020). Numerous bioactive substances, including as alkaloids, tannins, saponins, flavonoids, cardiac glycosides, and terpenoids, have been identified by phytochemical investigations (Akinyemi *et al*., 2005). These substances are known to have a part in the plant's various pharmacological actions, including those that are good for the heart.

Fig1.2 *Alstonia boonei* Growing in its natural habitat at University Benin, Benin City (UNIBEN)

1.3.1 JUSIFICATION FOR RESEARCH OF CARDIOVASCULAR POTENTIAL OF ALSTONIA BOONEI.

(a.) Rich Tradition in Ethnopharmacology

One of the most popular medicinal plants in West African ethnomedicine is *Alstonia boonei* , which is used to treat heart-related conditions like palpitations, chest discomfort, and hypertension. Its lengthy history of use by traditional healers in Sierra Leone, Ghana, and Nigeria offers a historically and culturally sound justification for biomedical research. While discovering new medicinal substances, recording and scientifically verifying traditional knowledge helps to preserve indigenous practices (Odugbemi, 2008; Sofowora *et al*., 2013).

(b.) Proven Vasodilatory and Hypotensive Effects

According to preclinical research, extracts of A. boonei in animal models, dramatically lowers blood pressure. These effects are thought to be caused by diuretic and vasorelaxation actions, which may be mediated by nitric oxide pathways or calcium channel blockage, Akinmoladun *et al*. (2020). It may be possible to create new classes of antihypertensive medications by looking into the precise processes underlying its hypotensive activity.

(c.) Numerous Phytochemicals That Protect the Heart

A. boonei 's phytochemical analysis are abundant in alkaloids, flavonoids, saponins, tannins, and terpenoids, all of which have been shown to have cardiovascular benefit. For example, flavonoids are known to have lipid-lowering, antioxidant, and vasoprotective qualities (Tapas *et al*., 2008). Examining these substances could result in the discovery of bioactive chemicals that have therapeutic use in the management or prevention of heart conditions.

(d.) Anti-inflammatory and Antioxidant Properties

The onset and advancement of cardiovascular disorders like atherosclerosis and heart failure are significantly influenced by oxidative stress and inflammation. Strong antioxidant activity has been demonstrated by *Alstonia boonei* extracts, which scavenge free radicals and lower lipid peroxidation (Erukainure *et al*., 2011). Its anti-inflammatory properties also point to the possibility of reducing endothelial dysfunction and vascular inflammation, Ajaiyeoba *et al*. (2003). These characteristics call for more pharmacodynamic and molecular research.

(e.) In Low-Income Areas, Affordability and Accessibility

Due to availability and cost, traditional cardiovascular medications are not widely available in many parts of Africa. A. Boonei is a cost-effective and locally accessible plant that offers a sustainable and socially acceptable substitute. Studies on its safety and effectiveness may result in the creation of easily accessible phytomedicines, which would lessen reliance on imported synthetic medications (Sofowora, 1993).

(f.) The necessity of standardization and scientific validation

Although it's widely used in conventional medicine, A. Boonei does not have recognized pharmacopoeial status, standardized dose forms, or comprehensive clinical investigations. Its medicinal claims remain anecdotal in the absence of a thorough scientific examination. Standardized formulations, ideal doses, toxicity profiles, and pharmacokinetics all crucial for safety and regulatory approval can be established with the aid of structured research (Kunle *et al*., 2012).

(g.) Possibility of Aiding in the Development of New Drugs

Natural goods continue to be a significant source of contemporary medications. Plants are the source of many cardiovascular medications, including reserpine from Rauwolfia serpentina and digitalis from Digitalis purpurea. *Alstonia boonei* may include special lead compounds with novel modes of action due to its complicated chemistry. Its bioactive compounds can be investigated by drug discovery programs for the creation of standardized cardiovascular treatments (Newman and Cragg, 2020).

(h.) Preservation of Biodiversity and Economic Growth Investigating and creating through sustainable cultivation and marketing of A. boonei, turning into medicinal goods encourages biodiversity conservation and provides financial incentives to nearby populations. The growing demand for herbal therapies worldwide has led to the validation of African medicinal plants such as A. Boonei has the potential to boost economic growth, especially in rural regions where herbal supply chains are active (WHO, 2019).

1.3.2 ALSTONIA BOONEI'S TOXICOLOGY PROFILE.

Assessing the safety of medicinal plants for human use requires an understanding of their toxicological profile. Despite being widely used in African traditional medicine to treat fever, pain, inflammation, and cardiovascular diseases, scientific research has shown that the safety of *Alstonia boonei* de Wild varies on the extract type, dosage, administration method, and length of usage. High doses or continuous use may present hazardous dangers to essential organs, even if typical doses are widely thought to be safe.

(a.) Acute Harm

The goal of acute toxicity studies is to ascertain a substance's safety threshold after a single or brief exposure. The acute toxicity of A. boonei extracts from bark and leaves in animal models has been evaluated in a number of investigations.

Example of Study: According to Akinmoladun *et al*. (2020), rats given ethanolic stem bark extract orally at doses up to 5000 mg/kg body weight did not die or exhibit any discernible behavioral abnormalities. According to OECD rules, this indicates a large margin of safety and categorizes the extract as practically non-toxic.

Extra Assistance:

After giving mice aqueous extracts of the bark at doses as high as 3000 mg/kg, Ajaiyeoba *et al.* (2003) likewise found no evidence of toxicity or death.

(b.) Chronic and Sub-Chronic Toxicology

The long-term consequences of repeated treatment are examined in sub-chronic and chronic toxicity studies, which is important for plants like A. boonei used for a long time.

Example of Study:

Over a 28-day period, Adebayo *et al.* (2016) assessed the sub-chronic toxicity of aqueous bark extract in Wistar rats. Histopathological analysis showed minor hepatic and renal changes, while liver enzymes (ALT and AST) showed modest rise at higher doses (1000 mg/kg). These results suggest that sustained high-dose exposure may cause hepatotoxicity and nephrotoxicity.

(c.) Toxicity Particular to Organs

Toxicological analyses of individual organs have revealed that A. boonei; When ingested in big quantities, may have mild to moderate effects on the kidneys and liver.

Kidney and Liver:

Rats' urea, creatinine, ALT, and AST levels changed in a dose-dependent manner, Erukainure *et al.* (2011), suggesting that the liver and kidneys are the main organs that are hazardous at high dosages.

The hematological system

Long-term use of methanol leaf extract changed hematological markers, such as hemoglobin levels and red blood cell counts, even at very high dosages, indicating possible hematotoxic consequences in unusual circumstances, Oladimeji *et al.* (2014).

(d.) Genotoxicity and Cytotoxicity

Research on A. Boonei still has few cytotoxic and genotoxic effects. Nonetheless, at therapeutic dosages, certain in vitro experiments indicate negligible harm in normal cell lines. Example of Study: According to Uboh *et al*. (2013), an aqueous leaf extract of A. In cultured human cells, boonei showed minimal cytotoxicity and did not result in notable chromosomal abnormalities, suggesting a somewhat safe genotoxic profile.

(e.) Human Usage Safety

Clinical evidence about *Alstonia boonei* 's toxicity in humans is scarce. However, given its lengthy history of traditional use in ethnomedicine and the paucity of known side effects, it appears to be generally well tolerated when taken in accordance with traditional dosage recommendations. However, the lack of carefully planned clinical studies necessitates prudence and regulatory oversight.

1.4.0 THE USE OF WISTAR RATS IN CARDIOVASCULAR RESEARCH.

One of the most popular laboratory animals in biomedical research, especially in the area of cardiovascular studies, are Wistar rats (Rattus norvegicus), an outbred breed of albino rat that was created in the early 20th century. Because of their genetic stability, repeatability, and physiological resemblance to human cardiovascular responses, their use is well-established.

(i.) Relevance to Physiology

The cardiovascular system of wistar rats is structurally and functionally similar to that of humans. They react similarly to several pharmacological drugs used to affect vascular tone, heart rate, and blood pressure, and their hearts have similar electrical characteristics (Kurtz and Morris, 1983). Because of their similarities, they are a suitable model for researching cardiovascular illnesses (CVDs), including heart failure, atherosclerosis, myocardial infarction, and hypertension.

(ii.) Screening for hypertension and antihypertensive drugs

In both induced and spontaneous models of hypertension, wistar rats are commonly employed. For example, deoxycorticosterone acetate (DOCA), Nω-Nitro-L-arginine methyl ester (L-NAME), and high-salt diets can all be used to chemically cause hypertension. Researchers can assess the effectiveness and mode of action of antihypertensive medications using these models, which mimic different types of human hypertension (Okoi-Ewa *et al.*, 2021). Because of their steady cardiovascular baseline and ease of handling during tests like non-invasive tail-cuff plethysmography or invasive pressure catheterization, wistar rats are also chosen when evaluating plant extracts (such as *Alstonia boonei*) for their potential to lower blood pressure.

(iii.) Research on Myocardial Infarction and Cardio-protection

Wistar rats are frequently employed in models of myocardial infarction (MI) brought on by doxorubicin toxicity, coronary artery ligation, or isoproterenol. According to Kumar *et al*. (2014), these models aid in evaluating the protective effects of novel medications or organic substances on cardiac tissues, enzymatic indicators, and histological alterations.

(iv.) Studies on Lipids and Atherosclerosis

Wistar rats can be used to research lipid metabolism and atherogenic processes by being fed diets high in fat or cholesterol, even though they are not predisposed to atherosclerosis by nature. This is helpful when assessing antiatherogenic and lipid-lowering medications (Bopanna *et al*., 1997).

(v.) Monitoring Hemodynamics and Vascular Research

Wistar rats are appropriate for invasive procedures such arterial catheterization, ECG recording, and vascular reactivity tests utilizing isolated organ pools because of their size and cooperative nature. To see how the test compound affects the cardiovascular system, researchers can take real-time measurements of the systolic, diastolic, and mean arterial pressure

(vi.) Safety and Assessment of Toxicology

Wistar rats are frequently used in toxicological evaluations associated with cardiovascular medications to track variables such heart weight, the histological integrity of cardiac tissues, electrocardiographic alterations, and blood pressure fluctuations. Early indicators of cardiotoxicity can be found in Wistar rats using sub-chronic toxicity tests (Olaleye *et al*., 2010).

1.4.1 BENEFITS OF USING WISTAR RATS ON CARDIOVASCULAR RESEARCH.

- Consistent cardiovascular reactions
- The simplicity of dietary and genetic modification
- Capability to adjust to different experimental settings
- The availability of physiological models that have been verified
- Baseline cardiovascular parameters that have been well documented

1.4.2 TYPICAL CARDIOVASCULAR EXPERIMENTAL MODELS.

Experimental models are used in cardiovascular disease (CVD) research to simulate human clinical circumstances, assess treatment options, and comprehend disease causes. Depending on the objectives of the study, these models which include ex vivo organ systems, in vitro cell cultures, and in vivo animal models, each have unique benefits.

1. Animal Models

(a.) Sprague-Dawley and Wistar Rats

Because of their availability, simplicity of handling, and predictable physiological responses, wistar rats are commonly employed in cardiovascular research. They are especially helpful in assessing how test chemicals affect cardiac histology, heart rate, and blood pressure (Okpo *et al*., 2016).

(b.) Rats with spontaneous hypertension (SHR)

Essential hypertension, which is similar to the human version of the disease, is genetically predisposed to develop in SHR models (Kurtz and Morris, 1983). They are frequently employed in research on hypertensive organ damage and antihypertensive medication screening.

(c.) Mice with ApoE−/− and LDLr−/−

These transgenic mouse models are ideal for studying atherosclerosis because they lack important proteins involved in lipid metabolism. Plaque and vascular inflammation are brought on by feeding them high-fat diets (Getz and Reardon, 2012).

(d.) Guinea Pigs and Rabbits

Because of their high vulnerability to diet-induced hyperlipidemia, rabbits are frequently utilized in cholesterol-fed models to research atherogenesis (Fan and Watanabe, 2000).

2. Induced Disease Models

(a.) Hypertension Induced by L-NAME

By preventing the generation of nitric oxide, L-NAME (Nω-nitro-L-arginine methyl ester) causes vasoconstriction and elevated blood pressure. This model aids in testing antihypertensive medications and investigating endothelial dysfunction (Félétou *et al*., 2018).

(b.) The DOCA-Salt Model

The DOCA-salt model mimics mineralocorticoid-induced hypertension by giving deoxycorticosterone acetate coupled with a high-salt diet, which causes volume-dependent hypertension and heart hypertrophy (Kurtz and Morris, 1983).

(c.) Myocardial Infarction Caused by Isoproterenol

Excessive dosages of the β-adrenergic agonist isoproterenol cause myocardial necrosis and oxidative stress, simulating a myocardial infarction in humans and enabling the assessment of cardioprotective medications (Kumar *et al*., 2014).

(d.) Ligation of the Coronary Arteries

This surgical model, which is frequently used to assess heart failure treatments, ligates the left anterior descending coronary artery in mice to produce a controlled myocardial infarction (Slezak *et al*., 1995).

(e.) Atherosclerosis Induced by a High-Fat Diet

When mice are fed a diet high in fat or cholesterol, their blood lipid levels rise and they develop early atherosclerotic abnormalities. Anti-inflammatory and lipid-lowering treatments are frequently tested using this paradigm (Zadelaar *et al*., 2007).

3. Models in Vitro and ex vivo

(a.) Assay for Isolated Aortic Rings

The vasodilatory or vasoconstrictive effects of substances on isolated rat or rabbit arteries can be tested using this vascular reactivity model (Adebayo and Ishola, 2009).

(b.) Making a Langendorff Heart

In order to evaluate contractility, coronary flow, and medication response without systemic effects, an ex vivo approach keeps the perfused heart outside the body (Bell *et al*., 2011).

(c.) Cell Lines of Cardiomyoblasts (H9c2)

For mechanistic research on cardiotoxicity, oxidative stress, and apoptosis, H9c2 cells obtained from rat embryonic ventricular tissue are frequently employed (Zhou *et al*., 2012).

d. Endothelial Cell Lines (HUVECs, for example)

In atherosclerosis models, human umbilical vein endothelial cells are essential for researching vascular inflammation, nitric oxide synthesis, and endothelial barrier function (Kraiss, 2005).

4. Model Zebrafish

Because zebrafish embryos are transparent and genetically tractable, the development of the heart and arteries can be seen in real time. Their application in high-throughput cardiovascular medication screening has grown (Lieschke and Currie, 2007).

5. Primates that are not humans (NHPs

Because of their striking anatomical and physiological similarities to humans, NHPs like macaques are employed in cutting-edge research, despite financial and ethical constraints (Cline *et al*., 2008).

1.4.3 ETHICAL CONSIDERATIONS OF ANIMAL RESEARCH

Understanding illness mechanisms and developing treatments has greatly benefited from the use of animals in scientific study, especially in biological domains like cardiovascular investigations.

Nonetheless, it brings up important moral issues pertaining to moral duty, scientific need, and animal welfare. Globally, ethical frameworks and laws have been put in place to address these issues and guarantee the responsible and compassionate use of animals in research.

(i.) The three Rs: Refinement, Reduction, and Replacement

Russell and Burch's (1959) 3Rs principle; Replacement, Reduction, and Refinement, is one of the most fundamental ethical principles in animal experimentation.

- Substitution entails the use of non-animal substitutes, such as invertebrate models, computer simulations, or cell cultures.

- The goal of reduction is to utilize as few animals as possible while still producing data that are statistically meaningful.

According to Festing and Wilkinson (2007), refinement is the process of altering experimental protocols to lessen discomfort, suffering, or distress.

This approach has been used in ethical review procedures all over the world to strike a balance between compassion and scientific research.

(ii.) Ethical and Scientific Rationale

Any use of animals in research must be scientifically justified. Researchers are required to demonstrate that the potential benefits of their work—such as new therapeutic discoveries or improved scientific understanding—significantly outweigh the possible risks to the animals involved. Ethical review bodies such as Animal Ethics Committees (AECs) and Institutional Animal Care and Use Committees (IACUCs) rigorously evaluate research protocols to ensure compliance with humane standards and ethical principles (Ormandy, Schuppli & Weary, 2009).

(iii.) Institutional and Legal Oversight

Animal research is governed by strict national and international regulations. In Europe, Directive 2010/63/EU outlines detailed requirements for the ethical use, care, and housing of laboratory animals (European Parliament, 2010). In the United States, the Animal Welfare Act (AWA) and the Public Health Service Policy provide the primary legal and ethical frameworks for animal experimentation (National Research Council, 2011).

Nigeria, like many other African countries, adheres to the National Code of Health Research Ethics, which includes provisions for animal welfare and responsible conduct in research (NHREC, 2007). Before any research can start, ethical approval is needed, and institutions are obligated to conduct routine inspections of animal facilities and procedures.

(iv.) Humane Treatment and Welfare

Positive experiences including healthy eating, environmental enrichment, and social contact are all included in the concept of animal wellbeing, in addition to the absence of pain and suffering (Fraser, 2008). Important ethical responsibilities during experimentation include pain control, humane outcomes, and routine health monitoring (Olsson *et al*., 2010).

(v.) Accountability and Transparency

For both scientific legitimacy and public trust, animal research must be transparent. To increase reproducibility and ethical accountability, guidelines like ARRIVE (Animal Research: Reporting of In Vivo Experiments) encourage accurate and thorough reporting of animal experiments (Kilkenny *et al*., 2010). Responsible research is reinforced by publishing both positive and negative results, revealing the number of animals used, and outlining humane procedures.

(vi.) Religious and Cultural Aspects

Different cultures and religions may have different ethical perspectives on animal research. For instance, certain cultures view particular species as having spiritual or symbolic meaning, which may influence whether or not people accept their employment in experimental settings (Reinhardt, 2002).

1.5.0 OBJECTIVES AND GOALS OF THE STUDY

The primary objective of this study is to examine the cardiovascular effects of *Alstonia boonei* using validated animal models. This investigation seeks to provide scientific support for its traditional use in the management of cardiovascular diseases such as hypertension, cardiac ischemia, and oxidative myocardial injury. *Alstonia boonei* is widely used in West African traditional medicine, particularly in Nigeria and Ghana, for treating heart-related disorders. Therefore, this study aims to explore the mechanisms of action, pharmacological properties, and safety profile of the plant (Agyare et al., 2013).

GOALS OF THE STUDY

1. To evaluate the effect of *Alstonia boonei* extract on nitric oxide bioavailability and endothelial function

Endothelial dysfunction is a common hallmark of many cardiovascular diseases. Evidence suggests that *A. boonei* may exert vasodilatory effects by enhancing nitric oxide synthesis or reducing oxidative stress, which impairs endothelial function (Adebayo & Ishola, 2009). This study will assess serum nitrate/nitrite levels and evaluate endothelium-dependent relaxation in isolated aortic rings to achieve this goal.

2. To elucidate the mechanisms by which *Alstonia boonei* influences cardiovascular regulation

The cardiovascular effects of medicinal plants often involve multiple biochemical pathways. Phytochemical compounds in *A. boonei*, such as alkaloids and flavonoids, may act on nitric oxide synthase (NOS), calcium channels, or the angiotensin-converting enzyme (ACE) (Oboh et al., 2014). To clarify the plant's mechanism of action, this study will explore receptor antagonism, calcium influx assays, and enzyme inhibition analyses.

3. To assess vascular responsiveness using isolated tissue models

Vascular reactivity studies in isolated rat aorta provide direct insight into the contractile or relaxant effects of plant extracts. Previous findings indicate that *A. boonei* induces concentration-dependent vasorelaxation in phenylephrine-precontracted tissues (Adebayo & Ishola, 2009). This study will measure both endothelium-dependent and endothelium-independent responses to determine the extract’s overall vascular effects.

4. To examine how *Alstonia boonei* extract affects cardiovascular parameters in relation to dose
The determination of therapeutic windows and safety margins requires a well-defined dose-response relationship. Graded doses of A. boonei will be administered in this trial to evaluate its effects on vascular tone, heart rate, and blood pressure pharmacodynamic profile (Okoi-Ewa *et al*., 2021). Standardization for upcoming clinical applications will be informed by data.

5. To look into *Alstonia boonei* 's antioxidant qualities in relation to cardiovascular protection
Endothelial dysfunction and vascular damage are significantly influenced by oxidative stress. In the heart and aortic tissues, the extract's capacity to scavenge free radicals and lessen lipid peroxidation will be assessed. According to Oboh *et al*. (2014) and Victor *et al*. (2020), this activity may assist maintain nitric oxide bioavailability and avoid vascular injury.

6. To assess the extract's cardiovascular safety and toxicity in vivo
Clinical translation must be preceded by a comprehensive safety evaluation. According to earlier research, *Alstonia boonei* is generally harmless at therapeutic dosages, but at larger concentrations, it may become harmful to particular organs (Olaleye *et al*., 2010). In rats given short- and long-term doses, this goal will track organ weights, hematological parameters, histology, and biomarkers of heart harm.

1.6.0 STATEMENTS OF RESEARCH CHALLENGES

1. Insufficient Empirical Support for Conventional Uses

Alstonia boonei has been used by traditional healers in West and Central Africa to cure heart conditions and hypertension, however these uses are largely based on oral tradition and cultural beliefs rather than scientific research. There is still a dearth of empirical support using in vitro and in vivo models. Burkill (1995) asserts that the therapeutic adaptability of A. Boonei has a strong ethnobotanical reputation, however there are still few contemporary pharmacological research on the plant, leaving a large knowledge vacuum that prevents scientific validation.

2. Uncertain Pharmacological Mechanism of Action

While initial research indicates that A. boonei, although may have vasodilatory and hypotensive effects (Ojewole, 2004), little is known about the pharmacological and molecular mechanisms behind these effects. Whether its effects are mediated by prostaglandin regulation, adrenergic modulation, calcium channel antagonism, or nitric oxide pathways has not been definitively established. Its development into a medicinal drug is speculative and lacks mechanistic support in the absence of clarity regarding its mode of action (Akinmoladun *et al*., 2010).

3. Lack of Preclinical Models Particular to the Heart

Understanding of the specific effects of A. boonei on circulatory physiology has been constrained by the limited use of animal models relevant to the cardiovascular system, such as nitric oxide synthase-inhibited models or spontaneously hypertensive rats (SHR). Few researchs evaluate its vascular effects or antihypertensive potential in disease-like situations using pathologically relevant models (Adeneye and Agbaje, 2007). The results' translational usefulness is weakened by the absence of a context-specific experimental design.

4. Absence of Pharmacokinetic Profiling and Standardized Dosing

Standardized dosage schedules and pharmacokinetic profiles for extracts from *Alstonia boonei* are not available. Different research has produced conflicting results due to differences in preparation techniques, plant parts employed, and extraction solvents (Yusuf *et al*., 2014). Reproducibility and dosage optimization for therapeutic use are still difficult to achieve in the absence of well-defined bioavailability, metabolism, half-life, or plasma concentration data.

5. Inadequate Knowledge of Vascular and Endothelial Impacts

Even Nevertheless, there is some evidence that supports, few researchs have comprehensively assessed the impact of A. boonei's vasorelaxant action on endothelial function or nitric oxide generation (Ajibade *et al*., 2013). A plant that has therapeutic claims in the field of cardiovascular disease must exhibit positive endothelium regulation because endothelial dysfunction is at the heart of many of these conditions. This field is still incredibly understudied and requires clarification using suitable vascular reactivity tests and molecular markers.

6. Limited Long-Term Safety Assessment and Toxicological Research

Toxicological information about A. Boonei is still fragmented, with the majority of studies focusing only on LD_{50} determination or acute toxicity. Data on genotoxicity, reproductive toxicity, organ-specific histopathology, and long-term exposure consequences are mostly lacking (Kuffuor *et al*., 2011). Its approval for clinical usage or pharmaceutical development is hampered by these shortcomings.

7. Potential for Drug Development Underutilized Despite Encouragering Phytochemistry

Even though several bioactive substances, including echitamine and derivatives of ursolic acid, have been identified, A. According to Ishola *et al*. (2016), boonei has not yet produced any authorized medicinal medicines. Its therapeutic potential has been underused due to a lack of integrated frameworks for pharmacological development and pharmacognostics. Its progress has also been hampered by a lack of cooperation between biomedical researchers and traditional healers.

CHAPTER TWO

MATERIALS AND METHODS

2.1.1 Materials

This research employed a range of materials grouped into biological specimens, laboratory glassware, and general laboratory equipment. These materials were critical in maintaining the accuracy, efficiency, and reliability of the experimental procedures.

2.1.2 Biological Materials

The principal biological specimen utilized in this study was the thoracic aorta extracted from Wistar rats. The tissue was isolated and prepared for the evaluation of vascular responses. In addition, a gas mixture comprising 95% oxygen and 5% carbon dioxide (Carbogen gas) was employed to aerate the Krebs-Henseleit solution, thereby ensuring adequate oxygenation and sustaining physiological pH conditions throughout the experiment.

Labouratory Glasswares

Various types of laboratory glassware were employed for solution preparation, sample handling, and experimental procedures. Each item served a specific purpose to ensure precision, safety, and accuracy throughout the experiment.

(a.) Volumetric Flasks (100 mL – 500 mL): Utilized for accurate preparation Conical and dilution of chemical and physiological solutions.

(b.) Beakers (25 mL – 1000 mL): Used for mixing and temporary storage of liquid solutions.
(c.) Measuring Cylinders (10 mL – 500 mL): Applied for precise measurement and transfer of liquid volumes.
(d.) Glass Funnels with Filter Paper: Used for filtering plant extracts and other solutions during the purification process.

“(e.) Petri Dishes: Used to hold and maintain tissue samples before mounting them in the organ bath for physiological assessment.

General Laboratory Supplies

A range of general laboratory supplies was employed to ensure sterility, safety, and precision during all experimental procedures. These materials were essential for maintaining a clean and controlled working environment.

(a.) Lab Coats and Safety Goggles: Provided protection against chemical splashes and potential biological exposure.

(b.) Disposable Gloves: Worn to prevent contamination while handling biological tissues, chemicals, and equipment.

(c.) Digital Weighing Balance: Used to accurately measure plant extracts, reagents, and other substance required for experimentation.

(d.) Parafilm: Utilized to seal containers securely, thereby preventing contamination and evaporation of solutions.

(e.) Tissue Papers and Wipes: Applied for cleaning laboratory surfaces and drying glassware and instruments after use.

(f.) Other Accessories: Included items such as spatulas, labels, and marker pens, which facilitated proper sample handling, organization, and documentation throughout the study.

2.1.3 Apparatus

Several laboratory instruments and equipment were employed in this study to support plant extraction, tissue preparation, experimental setup, and data acquisition. Each apparatus played a crucial role in ensuring precision, maintaining controlled experimental conditions, and achieving reliable measurements. The apparatus used included the following

Soxhlet Apparatus

In this research, a Soxhlet extraction device was employed to prepare ethanolic extracts from botanical materials. The Soxhlet apparatus is a tool used in labs to get soluble chemicals out of solid samples by using a continuous solvent reflux system. It works by letting the solvent, in this case ethanol, periodically percolate through the plant material. This makes sure that bioactive compounds are extracted efficiently without the need for significant amounts of solvent (Azwanida, 2015). To do this, you heat ethanol in a flask until it turns into gas. The gas then cools down and drips over the plant sample in a thimble. When the solvent in the thimble gets to a particular level, it flows back into the flask, taking the dissolved chemicals with it. This cycle goes on by itself until the extraction is done, resulting in a concentrated ethanolic extract that can be used for more testing (Luque de Castro & Priego-Capote, 2010).

Organ Bath System

(a.) Organ Bath (25–50 mL Capacity): Functioned as the chamber in which isolated aortic rings were immersed in the aerated Krebs-Henseleit solution, ensuring optimal physiological conditions for vascular response studies.

(b.) Aerator (Carbogen Gas Delivery System): Delivered a continuous gas mixture of 95% oxygen and 5% carbon dioxide to oxygenate the Krebs-Henseleit solution and maintain pH stability.
(c.) Water Bath (37°C): Maintained a constant temperature of 37°C to replicate physiological body conditions and sustain tissue viability during the experiment.

Data Recording and Measurement Instruments

(a.) Isometric Force Transducer: Detected and converted variations in mechanical tension within the aortic tissue into measurable electrical signals.

(b.) Physiograph: Displayed real-time graphical traces of tissue contractions and relaxations during the experiment.
(c.) Data Acquisition System (Computer Software): Recorded, processed, and analyzed the data generated, ensuring accurate interpretation of vascular responses.

Tissue Dissection and Handling Instruments

(a.) Scalpel with Blades: Utilized for precise excision and isolation of the thoracic aorta from surrounding tissues.

(b.) Scissors (Straight and Curved): Employed to trim and remove connective tissues, ensuring clean preparation of the aortic rings.

(c.) Forceps (Fine and Blunt-Tipped): Used to hold, manipulate, and position the aortic tissue carefully during dissection and mounting in the organ bath.

Equipment for Measuring with Precision

(a.) Micropipettes (0.1–1000 µL): Utilized for the exact measurement and transfer of very small liquid quantities, including medication preparations and plant extract solutions.

(b.) Graduated Pipettes: Employed for accurate liquid measurement and dilution during the creation of experimental and physiological solutions.

2.1.4 Chemicals and Drugs

A range of chemicals and reagents was applied in this investigation for the formulation of physiological solutions, activation of vascular contractions, and evaluation of the effects of *Alstonia boonei* on isolated rat aorta tissues. These drugs were critical for preserving experimental accuracy, assuring reproducibility, and getting valid pharmacological results.

Vasoactive Agents and Plant Extract:

Vasoactive agents were utilized to induce and quantify vascular responses in isolated aortic tissue. These agents modulate smooth muscle tone through various physiological mechanisms.

(a.) Potassium Chloride (KCl): KCl triggers contraction of vascular smooth muscle by depolarizing the cell membrane, resulting in calcium influx and subsequent muscular contraction.

(b.) Norepinephrine (NE): NE is a potent adrenergic agonist that activates α_1-adrenergic receptors on vascular smooth muscle, causing vasoconstriction through enhanced intracellular calcium mobilization.

Plant Extract:

The extract of *Alstonia boonei* was employed as the test compound to investigate its potential effects on vascular function. The extract was prepared in varying concentrations and administered in precise volumes to assess its pharmacological activity on the isolated aortic tissue.

Extract Concentrations:

A stock solution was prepared by dissolving 0.5000 g of dried *A. boonei* extract in 10 mL of distilled water. From this stock, five working concentrations were produced: 1.5625 mg/mL, 3.125 mg/mL, 6.25 mg/mL, 12.5 mg/mL, and 25 mg/mL. The extract was applied in corresponding volumes of 25 µL, 62.5 µL, 125 µL, 250 µL, and 500 µL to evaluate dose-dependent vasorelaxant effects (Adaramoye et al., 2005).

Physiological Salt Solution (PSS)

Physiological Salt Solution (PSS) was used to maintain the viability and contractile function of the isolated rat aorta throughout the experiments. The solution closely replicates the ionic composition of extracellular fluid, providing an optimal environment to ensure tissue responsiveness and stability during the study.

Composition of PSS:

The PSS contained key electrolytes and compounds necessary for maintaining normal cellular function and vascular tone:

Sodium chloride (NaCl): Maintains osmotic balance and regulates electrolyte distribution.

Potassium chloride (KCl): Regulates membrane potential and smooth muscle contraction.

Calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$): Provides calcium ions essential for excitation–contraction coupling in smooth muscle.

Magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$): Supports enzymatic activity and contributes to smooth muscle relaxation.

Sodium bicarbonate ($NaHCO_3$): Acts as a buffer to maintain physiological pH in the tissue bath.

Dextrose (glucose): Serves as the primary energy substrate for cellular metabolism.

Potassium dihydrogen phosphate (KH_2PO_4): Assists in buffering and supports intracellular metabolic processes.

Function of PSS

To preserve the functional integrity of the vascular tissue, the PSS was continuously aerated with carbogen gas (95% O_2 and 5% CO_2). This process maintained adequate oxygenation and stabilized pH throughout the experiment. The consistent physiological environment provided by the PSS ensured that all recorded vascular responses were accurate and reflective of true pharmacological activity of *Alstonia boonei* extract.

2.2.0 Methods

2.2.1 Preparation of Physiological Salt Solution (PSS)

The PSS was prepared following a standardized procedure to ensure complete solubility of all components and to maintain the physiological balance of ions necessary for the isolated rat aorta.

Preparation Procedure

1. Dissolution of Main Components

A measured volume of distilled water was transferred into a clean beaker.

The following reagents were gradually added one after the other, ensuring complete dissolution before adding the next:

Sodium chloride ($NaCl$) – 6.9 g/L

Sodium bicarbonate ($NaHCO_3$) – 2.1 g/L

D-glucose – 2.0 g/L

Potassium dihydrogen phosphate (KH_2PO_4) – 0.16 g/L

Potassium chloride (KCl) – 0.36 g/L

Magnesium sulfate monohydrate ($MgSO_4 \cdot H_2O$) – 0.29 g/L

2. Preparation of Calcium Chloride Solution

Calcium chloride ($CaCl_2$) – 0.37 g/L was dissolved separately in a small volume of distilled water.

This step prevented precipitation that could occur when calcium is mixed directly with phosphate or bicarbonate-containing solutions.

The fully dissolved calcium chloride solution was then added gradually to the main mixture with continuous stirring.

3. Aeration of the Solution

2.2.2 Aeration and Sbtorage of Physiological Salt Solution (PSS)

The prepared PSS was continuously aerated with carbogen gas (95% O_2, 5% CO_2) to maintain adequate oxygenation and stabilize pH, ensuring compatibility with the isolated vascular tissue.

Storage and Usage:

The freshly prepared PSS was stored at room temperature and used immediately during experiments to preserve ionic balance and freshness.

For longer experimental sessions, the solution was maintained under continuous carbogen aeration to sustain physiological pH, prevent precipitation, and preserve overall effectiveness throughout the duration of the study.

2.2.3 Collection and Preparation of *Alstonia boonei* Leaves

Leaves of *Alstonia boonei* were collected from the vicinity of the Faculty of Pharmacy, University of Benin, Benin City. Plant identification and authentication were confirmed by a plant taxonomist from the Pharmacognosy Department, University of Benin, Nigeria.

Drying and Grinding: Collected leaves were shade-dried at ambient room temperature to preserve heat-sensitive phytoconstituents. Drying continued until a constant weight was achieved, confirming complete removal of moisture. The dried leaves were then ground into a fine powder using a mechanical grinder to increase surface area and facilitate efficient solvent extraction. The powdered sample was stored in an airtight container until further use (Oyebode et al., 2019).

Extraction Procedure:

Bioactive compounds were extracted using a Soxhlet apparatus. A 100 g portion of the powdered leaves was placed in a Soxhlet thimble, and ethanol was employed as the extraction solvent. The extraction process lasted six hours, during which the solvent was repeatedly vaporized and condensed to wash the plant material thoroughly. Following extraction, ethanol was removed via rotary evaporation to obtain the crude ethanolic extract. The extract was stored in a cool, dry place in an airtight container until further analysis (Olajide et al., 2015).

2.2.4 Preparation of *Alstonia boonei* Extract Solutions

Stock Solution Preparation:

Weighing: Using a precision digital balance, 0.5 g of dried *A. boonei* extract was accurately weighed to ensure consistent dosing (Omoya & Oyebola, 2019).

Dissolution: The extract was dissolved in 10 mL of distilled water, stirred continuously with a magnetic stirrer until a clear, homogenous solution was obtained.

Preparation of Working Concentrations:

Preparation, Dilution, and Storage of Extract Solutions:

The stock solution of *Alstonia boonei* extract was serially diluted with distilled water to achieve the desired experimental concentrations: 1.5625, 3.125, 6.25, 12.5, 25, and 50 mg/mL. Appropriate volumes of the stock solution were combined with distilled water to ensure precise concentration gradients for all experimental trials (Omoya & Oyebola, 2019).

Volume Measurement and Storage:

For each concentration, exact volumes (25 µL, 62.5 µL, 125 µL, 250 µL, and 500 µL) were measured using a micropipette to guarantee consistency across trials. The prepared solutions were stored at room temperature and used immediately after preparation to maintain the stability and bioactivity of the phytochemical constituents (Omoya & Oyebola, 2019).

2.2.5 Experimental Animals

Selection and Justification:

Wistar rats are frequently employed in studies of vascular reactivity, hypertension, and vasorelaxant activity due to their anatomical and physiological similarities to the human cardiovascular system (Adebayo et al., 2019). Healthy adult male rats, weighing 180–250 g, were selected to minimize hormonal fluctuations and physiological variability that could influence vascular responsiveness (Oyebola, 2021).

Housing and Care:

The animals were housed in standard laboratory cages with sufficient bedding and maintained under controlled environmental conditions, following recommendations of the National Research Council (NRC, 2011). Temperature, humidity, and ventilation were kept within optimal ranges, and a 12-hour light/dark cycle was observed to support normal circadian rhythms. Rats were provided with ad libitum access to food and water, and their health and wellbeing were monitored daily throughout the study.

Ethical Considerations:

All procedures involving Wistar rats were conducted in strict accordance with NRC (2011) guidelines to ensure ethical treatment. The study adhered to the principles of Replacement, Reduction, and Refinement (3Rs), aiming to minimize animal suffering while generating reliable scientific data. Animals were handled with care, observed routinely for signs of distress or illness, and any procedure with potential discomfort was justified and performed using methods to reduce pain. Ethical oversight was maintained throughout to ensure compliance with internationally recognized standards for laboratory animal care.

2.2.6 Experimental Procedure

1. Animal Preparation**:**

Adult Wistar rats weighing 180–250 g were selected due to their well-characterized cardiovascular responses, making them a standard model for vascular studies (National Research Council, 2011; Oliveira et al., 2020). The animals were acclimatized for seven days under standard laboratory conditions with unrestricted access to food and water.

2. Tissue Preparation:

Following humane sacrifice, the thoracic aorta was carefully excised and cleared of surrounding connective tissue. The aorta was immediately placed in cold Physiological Salt Solution (PSS) to maintain tissue viability and then cut into rings approximately 3–4 mm in length. The endothelium was preserved in all preparations to allow assessment of endothelium-dependent vascular responses (Furchgott & Zawadzki, 1980).

3. Mounting the Tissue

Aortic rings were mounted in a 25 mL organ bath containing PSS maintained at 37 °C. The bath solution was continuously aerated with carbogen (95% O_2, 5% CO_2), and each ring was connected to a force transducer linked to a data acquisition system for real-time monitoring of vascular tension (Furchgott and Zawadzki, 1980).

4. Stabilization

Mounted tissues were allowed to equilibrate for 60 minutes to ensure stable baseline tension. During this period, the PSS was replaced every 15 minutes to maintain optimal physiological conditions (Ribeiro Campos *et al*., 2018; Mahmoud *et al*., 2013).

5. Application of Extract

After stabilization, cumulative concentrations of *Alstonia boonei* extract were applied to the aortic rings. The extract concentrations were 1.5625, 3.125, 6.25, 12.5, 25, and 50 mg/mL, and corresponding volumes of 25 µL, 62.5 µL, 125 µL, 250 µL, and 500 µL were added sequentially at 4-

minute intervals. Vascular responses were recorded and analyzed for each concentration (Adaramoye *et al.*,2005).

2.2.7 Effect of the Extract on Intact Endothelium Aorta

To study the endothelium-dependent effects of *Alstonia boonei* extract, isolated thoracic aorta rings with intact endothelium were employed. This allows measurement of vascular relaxation mediated by endothelial factors. The study assessed how cumulative concentrations of the extract alter aortic tone. The following steps describe the experimental process in detail:

1. Tissue Preparation

Thoracic aorta was extracted from mature Wistar rats weighing 180–250 g following humane sacrifice. Connective tissue and fat were carefully removed, and the aorta was sliced into rings measuring roughly 3–4 mm in length. The endothelium was maintained intact to facilitate measurement of endothelium-dependent responses. The rings were immediately immersed in cold, aerated Physiological Salt Solution (PSS) to sustain tissue viability (Furchgott and Zawadzki, 1980; Ribeiro Campos *et al.*, 2018).

2. Stabilization

The aortic rings were mounted in a 25 mL organ bath containing PSS at 37 °C, constantly aerated with carbogen (95% O_2, 5% CO_2). Each ring was attached to a force transducer for real-time tension measurement. The tissues were allowed to equilibrate for 60 minutes, with the bath solution refilled every 15 minutes to establish stable baseline conditions before extract administration (Ribeiro Campos *et al.*, 2018; Mahmoud *et al.*, 2013).

3. Application of the Extract

After stabilization, cumulative doses of *Alstonia boonei* extract (1.5625, 3.125, 6.25, 12.5, 25, and 50 mg/mL) were administered. Corresponding volumes of 25 µL, 62.5 µL, 125 µL, 250 µL, and

500 µL were added consecutively at 4-minute intervals to allow sufficient tissue reaction at each dose (Adaramoye *et al.*, 2005).

4. Observation and Recording

Changes in vascular tension were continuously monitored using a data gathering device attached to the force transducer. Relaxation responses were expressed as a percentage of the initial pre-contracted tone. Observations focused on the degree and pattern of relaxation to detect endothelium-dependent effects of the extract, which were later examined statistically (Ribeiro Campos *et al.*, 2018; Mahmoud *et al.*, 2013).

2.2.8 Effect of *Alstonia boonei* Extract on KCl-Induced Contraction

The effect of *Alstonia boonei* extract on potassium chloride (KCl)-induced contractions in isolated rat aortic rings was investigated to assess its direct action on vascular smooth muscle, independent of endothelial contributions. High extracellular potassium depolarizes the smooth muscle membrane, activating voltage-dependent calcium channels and triggering contraction.

1. Tissue Preparation

Thoracic aorta segments were harvested from adult Wistar rats (180–250 g) following humane euthanasia. Surrounding connective tissue and fat were carefully removed, and the aorta was cut into rings approximately 3–4 mm in length. The endothelium was either preserved or removed according to the experimental design. The rings were immediately placed in cold, aerated Physiological Salt Solution (PSS) to maintain tissue viability (Furchgott & Zawadzki, 1980; Ribeiro Campos et al., 2018).

2. Stabilization

Aortic rings were mounted in 25 mL organ baths containing PSS at 37 °C, continuously aerated with carbogen gas (95% O_2, 5% CO_2). Each ring was attached to an isometric force transducer to measure changes in vascular tension. Tissues were allowed to equilibrate for 60 minutes, with the bath solution refreshed every 15 minutes to establish a stable baseline tension (Ribeiro Campos et al., 2018; Mahmoud et al., 2013).

3. Induction of Contraction and Extract Application

Following stabilization, aortic rings were pre-contracted with 80 mM KCl to induce maximal smooth muscle contraction. Once a steady contraction was achieved, cumulative concentrations of *Alstonia boonei* extract (1.5625, 3.125, 6.25, 12.5, 25, and 50 mg/mL) were administered. Corresponding volumes of 25 µL, 62.5 µL, 125 µL, 250 µL, and 500 µL were added sequentially at 4-minute intervals to assess the dose-dependent relaxing effect of the extract (Adaramoye et al., 2005).

4. Observation and Data Recording

Changes in vascular tension were continuously monitored using a data acquisition system connected to the force transducer. Relaxation responses were expressed as a percentage of the initial KCl-induced contraction, allowing evaluation of the extract's direct smooth muscle-relaxant effect, independent of endothelium-mediated mechanisms (Ribeiro Campos et al., 2018; Mahmoud et al., 2013).

2.2.9 Effect of *Alstonia boonei* Extract on Norepinephrine-Induced Contraction

The effect of *Alstonia boonei* extract on norepinephrine (NE)-induced contraction in isolated rat aortic rings was investigated to determine its ability to induce smooth muscle relaxation via receptor-mediated mechanisms. Both endothelium-intact and endothelium-denuded rings were used to differentiate between endothelium-dependent and independent effects.

1. Tissue Preparation

Thoracic aorta segments were harvested from adult Wistar rats (180–250 g) following humane euthanasia. Connective tissue and fat were carefully removed, and the aorta was sectioned into rings approximately 3–4 mm in length. The endothelium was either preserved or removed by gentle rubbing with a fine wire, according to the experimental design. Rings were immediately immersed in cold, aerated Physiological Salt Solution (PSS) to maintain tissue viability (Furchgott & Zawadzki, 1980; Ribeiro Campos et al., 2018).

2. Stabilization

Aortic rings were mounted in 25 mL organ baths containing PSS at 37 °C and continuously aerated with carbogen gas (95% O_2, 5% CO_2). Each ring was attached to an isometric force transducer, and tissues were allowed to equilibrate for 60 minutes. The bath solution was refreshed every 15 minutes to establish a stable baseline tension (Ribeiro Campos et al., 2018; Mahmoud et al., 2013).

3. Application of Norepinephrine and Extract

After stabilization, aortic rings were pre-contracted with 500 µL of 2 mg/mL norepinephrine to induce receptor-mediated contraction. Once a steady contraction was achieved, cumulative concentrations of *Alstonia boonei* extract (1.5625, 3.125, 6.25, 12.5, 25, and 50 mg/mL) were administered sequentially. Corresponding volumes of 25 µL, 62.5 µL, 125 µL, 250 µL, and 500 µL were added at 4-minute intervals to evaluate the extract's vasorelaxant effect.

4. Observation and Data Recording

Changes in vascular tension were continuously recorded using a data acquisition system connected to the force transducer. Relaxation responses were expressed as a percentage of the initial NE-induced contraction. Comparisons between endothelium-intact and denuded rings allowed determination of endothelium-dependent versus independent mechanisms of relaxation.

CHAPTER THREE

RESULTS

The extract of *Alstonia boonei* produced a clear concentration-dependent relaxation in the endothelium-intact thoracic aorta. As the concentrations increased from 1.5625, 3.125, 6.25, 12.5,25, 50 mg/mL, the vascular smooth muscle showed a progressive reduction in tension, indicating that higher doses of the extract exert stronger vasorelaxant effects. This graded response suggests that the bioactive constituents of *Alstonia boonei* act in a dose-responsive manner to reduce contractile tone, likely by enhancing endothelium-mediated relaxation pathways and/or limiting calcium availability within the smooth muscle. Overall, the result confirms that *Alstonia boonei* possesses an effective vasodilatory action that becomes more pronounced with increasing concentration.

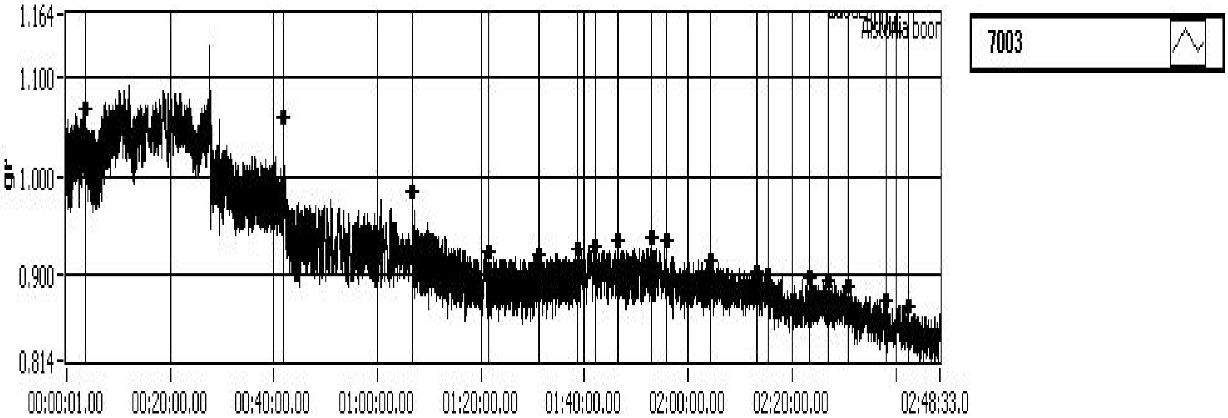

Fig 3.1 Relaxation effect of *Alstonia boonei* extract on endothelium-intact rat thoracic aorta, showing concentration-dependent relaxation of vascular smooth muscle.

The graphical representation of the mean vascular responses to the log-transformed doses of *Alstonia boonei* extract demonstrates a clear dose-dependent vasorelaxant effect on the endothelium-intact rat thoracic aorta. Beginning from the PSS baseline, the tension gradually decreases as the log dose increases from –0.973 to 0.436 mg/mL, indicating progressive relaxation of the vascular smooth muscle. This downward trend reflects the ability of the extract to attenuate vascular tone in a concentration-dependent manner, suggesting activation of endothelium-mediated pathways or direct smooth-muscle relaxation mechanisms. The error bars show minimal variability across doses, further supporting the consistency of the extract's vasorelaxant action. This highlights that increasing concentrations of *Alstonia boonei* reliably reduce vascular tension, reinforcing its potential role as a natural vasodilatory agent.

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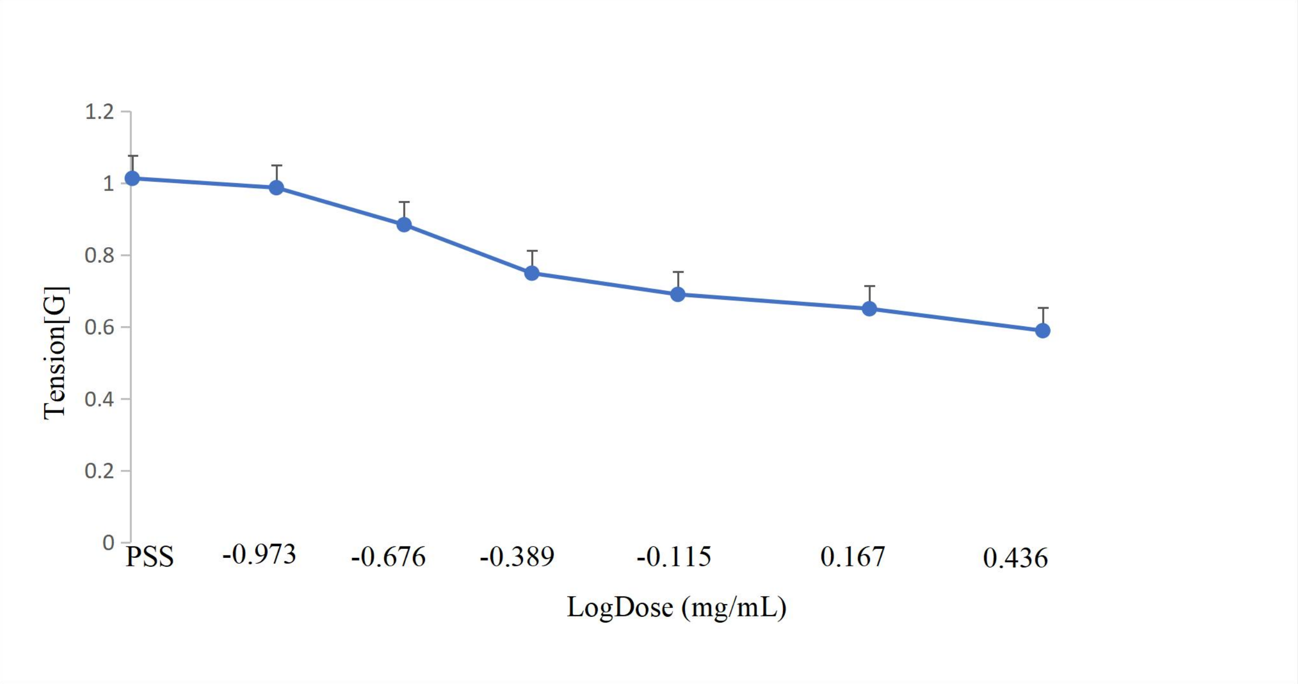

Fig 3.2 A graphical representation of the mean values of vascular response to the log dose value of the different concentration of *Alstonia boonei* extract on endothelium-intact rat thoracic aorta,

Where n = 6,,

This illustrates the contractile response of the rat thoracic aorta to KCl-induced depolarization, followed by the effect of applying the same concentration of *Alstonia boonei* extract to the precontracted tissue. At the introduction of KCl, there is an immediate and sharp rise in vascular tension, indicating successful membrane depolarization and activation of voltage-dependent calcium channels, which produced a strong, sustained contraction. Once a stable plateau of contraction was achieved, administration of the extract elicited a gradual but continuous reduction in vascular tension. This downward slope reflects a vasorelaxant effect of the extract on the depolarized smooth muscle. The relaxation despite persistent high extracellular potassium suggests that the extract may exert its effect through mechanisms not fully dependent on membrane hyperpolarization, possibly involving inhibition of calcium influx, interference with intracellular calcium mobilization, or modulation of contractile machinery. Overall, the tracing demonstrates that *Alstonia boonei* extract effectively attenuates KCl-induced contraction, supporting its potential calcium-antagonistic or direct smooth muscle–relaxing properties.

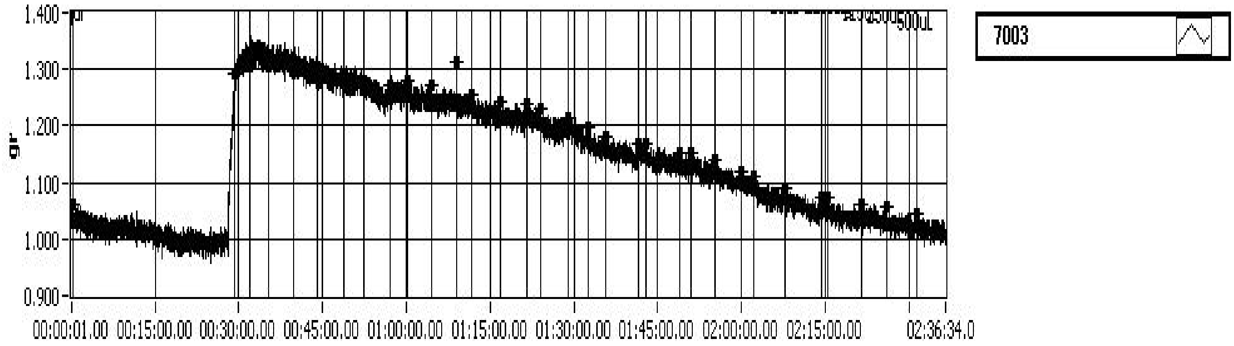

Fig 3.3 Relaxation Effect of *Alstonia boonei* extract on Potassium Chloride (KCL) induced contraction, showing concentration-dependent relaxation of vascular smooth muscle.

The graph shows the effect of cumulative log-dosed concentrations of *Alstonia boonei* extract on KCl-precontracted endothelium-intact rat aortic rings. As expected, KCl produced a strong initial contraction due to membrane depolarization and calcium influx through voltage-dependent calcium channels. With the introduction of the extract at increasing log doses (−0.97, −0.68, −0.39, −0.12, 0.17, 0.44 mg/ml), a steady, concentration-dependent decrease in vascular tension was observed, falling from about 1.38 g toward approximately 0.97 g at the highest dose. This progressive relaxation indicates that *A. boonei* effectively reduces KCl-induced contraction, suggesting a calcium-antagonistic mechanism; likely through inhibition of calcium entry or modulation of intracellular calcium handling. The pattern supports the extract's vasorelaxant potential and its relevance in cardiovascular regulation.

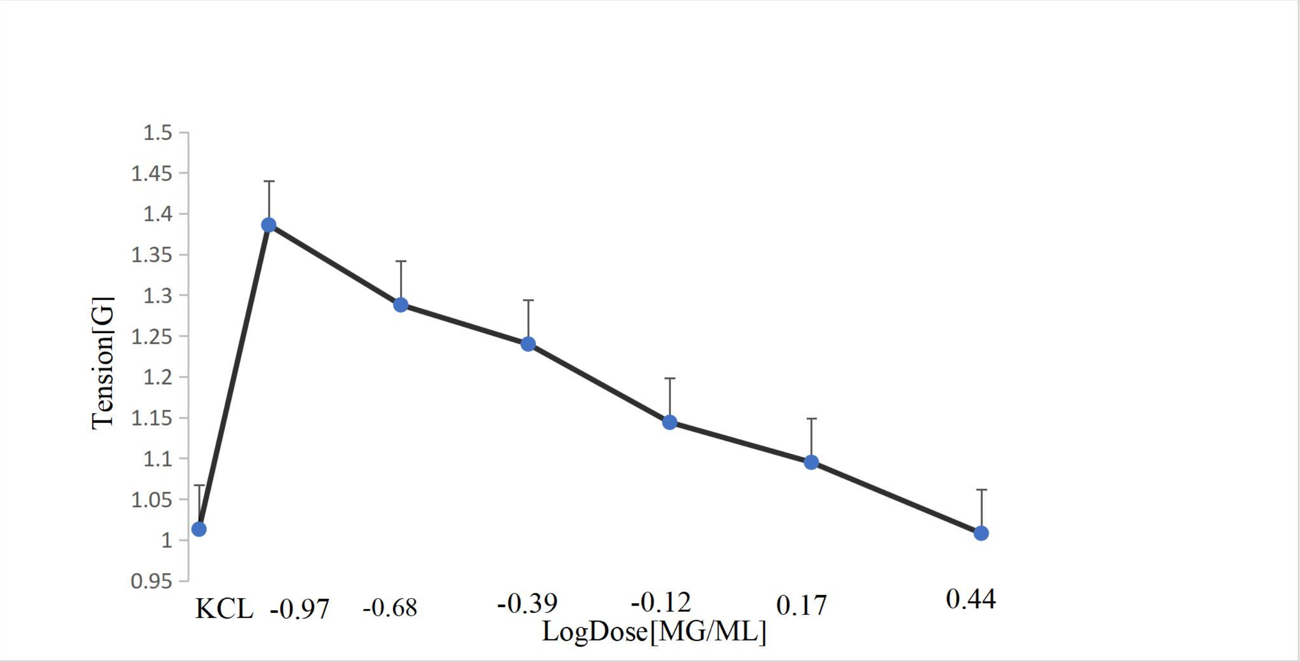

Fig 3.4 A graphical representation of the mean values of vascular response to the log dose value of the different concentration of *Alstonia boonei* extract on Potassium Chloride (KCL) induced contraction.

Where n = 6

The tracing shows that norepinephrine produced a rapid and sustained contraction of the endothelium-intact rat thoracic aorta, indicated by the sharp rise in tension following its administration. After reaching its peak, the contraction remained stable for a short period before gradually decreasing as incremental doses of *Alstonia boonei* extract were applied. Each subsequent dose of the extract produced a progressive fall in vascular tension, demonstrating a clear relaxant effect against norepinephrine-induced contraction. This pattern suggests that the extract effectively antagonizes norepinephrine-mediated vasoconstriction in a concentration-dependent manner, likely through interference with α-adrenergic signaling or enhancement of endothelial vasodilatory mechanisms.

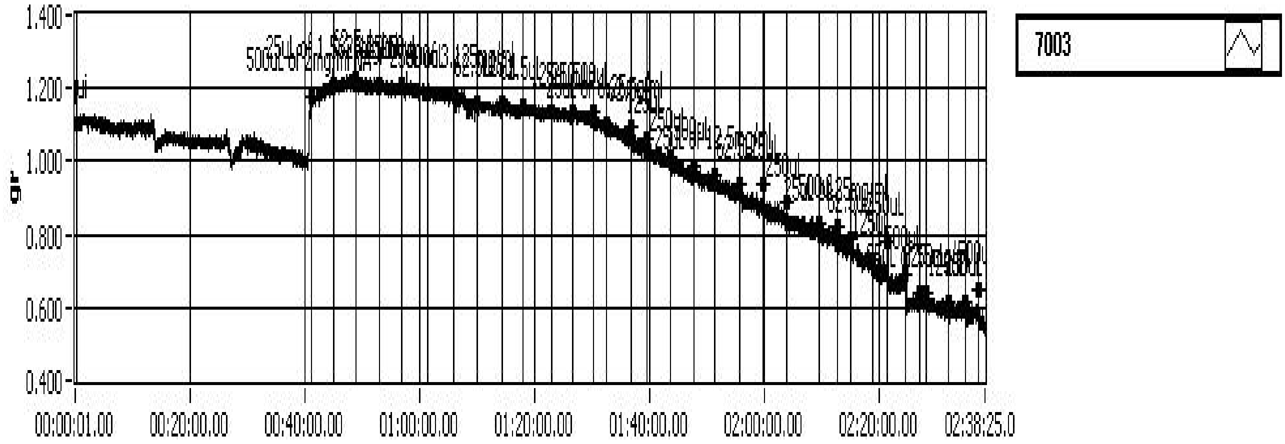

Fig 3.3 Relaxation Effect of *Alstonia boonei* extract on Norepinephrine-induced contraction, showing concentration-dependent relaxation of vascular smooth muscle.

The graph of norepinephrine-induced contraction shows that the addition of norepinephrine (NE) produced a sharp rise in vascular tension relative to the baseline physiological salt solution (PSS), confirming a strong α-adrenergic–mediated contraction of the endothelium-intact aortic rings. However, once the *Alstonia boonei* extract was applied in increasing concentrations (log doses −0.973 to 0.436 mg/ml), a clear, progressive decline in tension was observed. This downward trend indicates a dose-dependent vasorelaxant effect of the extract against NE-induced contraction. At lower concentrations, the relaxation is moderate, but as the dose increases, the extract consistently reduces vascular tension, eventually bringing it close to or below baseline levels. This pattern suggests that *Alstonia boonei* effectively antagonizes norepinephrine's contractile action, possibly through inhibition of calcium influx or interference with α-adrenergic signaling, demonstrating its potential as a vasoactive, relaxation-inducing agent.

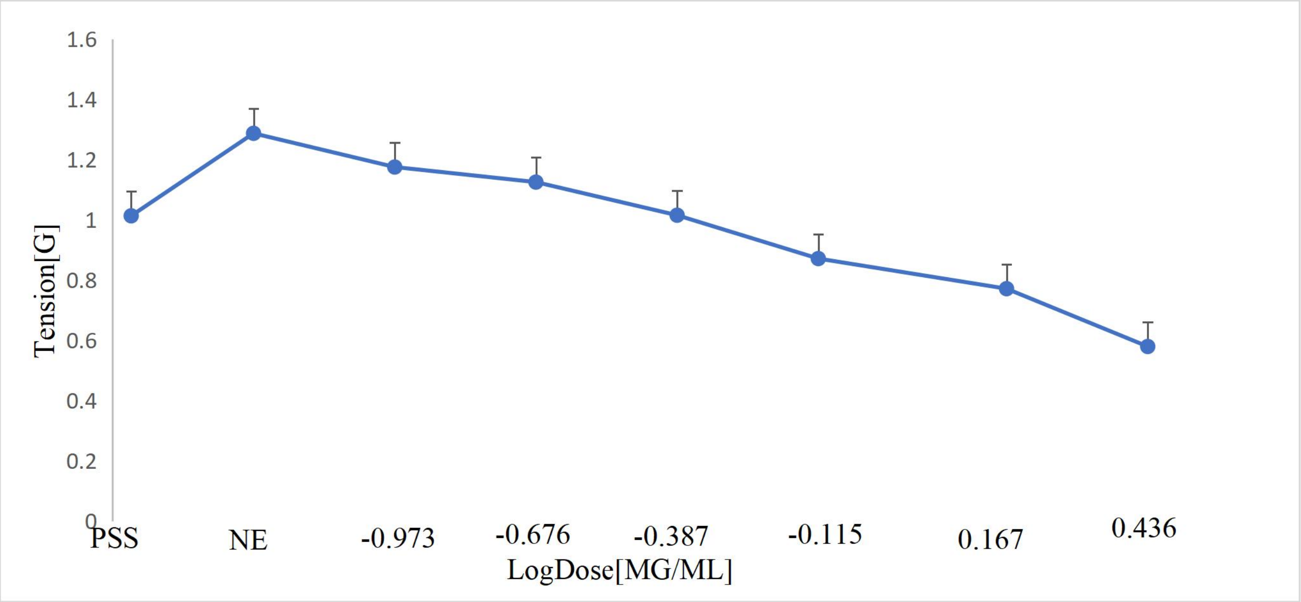

Fig 3.4 A graphical representation of the mean values of vascular response to the log dose value of the different concentration of *Alstonia boonei* extract on Norepinephrine-induced contraction.

Where n = 3

CHAPTER FOUR

DISCUSSION

4.1.1 The Effect of the Extract on Endothelium-Intact Thoracic Aorta

The graph shows a gradual and progressive reduction in vascular tension following the cumulative administration of *Alstonia boonei* extract at increasing concentrations (1.5625 mg/mL to 50 mg/mL). In the endothelium-intact aortic rings, the extract produced a dose-dependent vasorelaxation, indicated by the downward shift of the tension trace after each administered volume. The relaxation response became more pronounced as the concentration of the extract increased, suggesting that the extract contains bioactive constituents capable of modulating vascular tone. The presence of an intact endothelium is significant because it supports the involvement of endothelium-derived relaxing factors, particularly nitric oxide (NO), in mediating the vasodilatory effect. Many plant-based phytochemicals, especially flavonoids and alkaloids commonly found in *A. boonei*, are known to stimulate endothelial NO synthase (eNOS), leading to increased-NO release and subsequent smooth muscle relaxation (Gilani and Rahman, 2005; Duarte *et al*., 1993). This mechanism aligns with the observation that relaxation occurred smoothly and progressively rather than abruptly. The decline in contractile force throughout the recording suggests that the extract may work by enhancing NO availability, inhibiting calcium influx, or modulating receptor-mediated signaling pathways involved in vascular contraction. This result confirms that *Alstonia boonei* possesses endothelium-dependent vasorelaxant properties, supporting its traditional use in the management of cardiovascular-related conditions such as hypertension.

4.1.1 The Effect of the Extract on KCl-Induced Contraction

The recorded trace shows an initial sustained contraction of the aortic ring following exposure to 80 mM potassium chloride (KCl), which is consistent with KCl-induced membrane depolarization. High extracellular K^+ causes opening of voltage-dependent L-type Ca^{2+} channels, leading to calcium influx and smooth muscle contraction (Karaki and Weiss, 1988). Once contraction was stabilized, cumulative addition of *Alstonia boonei* extract resulted in a progressive and concentration-dependent relaxation of the vascular tissue, indicated by the gradual downward slope of the tension trace. This relaxation in the presence of sustained depolarization suggests that the extract exerts its vasorelaxant effect at least partly through inhibition of calcium entry into the smooth muscle, rather than acting solely through endothelial pathways. The response pattern indicates that the extract may block voltage-dependent Ca^{2+} channels or reduce intracellular Ca^{2+} mobilization, thereby attenuating contractile force (Kitagawa *et al.*, 1995). Because KCl-induced contraction is endothelium-independent, the ability of the extract to reverse this contraction supports the presence of direct smooth muscle relaxant activity, likely attributable to phytochemical constituents such as alkaloids, triterpenoids, flavonoids, and saponins commonly reported in *A. boonei* (Ojewole, 2002). The gradual and sustained nature of the relaxation also suggests that the extract may not act as an immediate antagonist but rather interferes with calcium signaling mechanisms regulating vascular tone. The result demonstrates that *Alstonia boonei* possesses calcium channel-modulatory vasorelaxant properties, reinforcing its traditional application in managing hypertension and vascular tension disorders.

4.1.2 The Effect of the Extract on Norepinephrine-Induced Contraction

When the thoracic aortic rings were pre-contracted with 500 µL of 2 mg/mL norepinephrine, a sustained increase in contractile tension was observed, indicating successful activation of α_1-adrenergic receptors on vascular smooth muscle. Norepinephrine produces contraction primarily through receptor-mediated intracellular signaling involving inositol triphosphate (IP_3) and subsequent release of stored Ca^{2+} from the sarcoplasmic reticulum, as well as facilitation of extracellular Ca^{2+} influx. Following the cumulative administration of *Alstonia boonei* extract, there was a gradual and concentration-dependent relaxation of the norepinephrine-induced contraction.

This downward shift in the tension trace demonstrates that the extract is capable of reversing receptor-mediated vasoconstriction.

The ability of the extract to attenuate norepinephrine-induced contraction suggests that its vasorelaxant activity is not limited to endothelium-mediated nitric oxide release alone, but also involves direct effects on the vascular smooth muscle. The relaxation may be due to inhibition of Ca^{2+} influx, suppression of intracellular Ca^{2+} mobilization, or interference with α_1-adrenergic signaling pathways responsible for sustaining contraction. This finding aligns with earlier reports that phytochemicals present in *Alstonia boonei* , including alkaloids, saponins and flavonoids, possess smooth muscle relaxing and calcium channel-modulating properties, which contribute to its traditional use in the management of hypertension and circulatory disorders. The observed relaxation of norepinephrine-induced contraction confirms that *Alstonia boonei* extract exerts a functional inhibitory effect on agonist-stimulated vascular tone, reinforcing its vasodilatory potential and supporting its pharmacological relevance as a natural antihypertensive agent.

CHAPTER FIVE

CONCLUSION, CONTRIBUTION TO KNOWLEDGE and RECOMMENDATION

5.1.1 CONCLUSION

The findings of this study demonstrate that the *Alstonia boonei* leaf extract possesses significant vasorelaxant activity on isolated thoracic aorta rings of Wistar rats. The extract produced a concentration-dependent relaxation in both endothelium-intact and endothelium-denuded aortic tissues, indicating that its mechanism of action involves both endothelial-dependent and direct smooth muscle effects. The relaxation observed in KCl-induced contraction suggests that the extract is capable of inhibiting calcium influx through voltage-dependent calcium channels, while the attenuation of norepinephrine-induced contraction indicates interference with receptor-mediated intracellular calcium mobilization. These combined observations imply that the vasodilatory effect of the extract is likely mediated through modulation of vascular calcium handling, as well as possible enhancement of nitric oxide bioavailability where the endothelium is intact. The results are consistent with the reported ethnomedicinal use of *Alstonia boonei* in the management of hypertension and related cardiovascular conditions.

5.1.2 CONTRIBUTION TO KNOWLEDGE

This study provides new evidence that *Alstonia boonei* possesses significant vasorelaxant activity on isolated rat thoracic aorta, demonstrating clear, concentration-dependent relaxation in tissues precontracted with KCl and norepinephrine. The ability of the extract to reverse KCl-induced contraction suggests a calcium-antagonistic mechanism, likely involving inhibition of voltage-dependent calcium influx. Its relaxation of norepinephrine-induced contraction further indicates possible interference with receptor-mediated or intracellular calcium pathways. Together, these findings scientifically validate the plant's traditional use in cardiovascular management and offer experimental support for its potential as a natural antihypertensive agent. The results also contribute baseline pharmacological data on extract concentrations, vascular responses, and mechanistic implications, forming a foundation for future studies on bioactive constituents, safety evaluation, and therapeutic formulation.

5.1.3 RECOMMENDATION

Based on the observed concentration-dependent vasorelaxation of *Alstonia boonei* extract on both KCl- and norepinephrine-induced contractions, it is recommended that further studies be carried out to isolate, characterize, and quantify the specific bioactive compounds responsible for the vascular relaxation. Additional mechanistic studies using calcium-free solutions, receptor blockers, and endothelial inhibitors are advised to clearly define whether the extract acts through calcium channel blockade, endothelial nitric oxide pathways, or receptor antagonism. Long-term toxicity and in-vivo antihypertensive studies should also be conducted to establish safety and therapeutic potential. Finally, standardization of extract preparation and dosing is recommended to support future pharmacological development and possible clinical application.

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