# EFFECT OF METHANOL LEAF EXTRACT OF *Anthocleista grandiflora* ON KIDNEY FUNCTION

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**BENIN CITY**

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**AN UNDERGRADUATE PROJECT WORK SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA; IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR AWARD OF BACHELOR OF SCIENCE (B.SC.) DEGREE IN SCIENCE LABORATORY TECHNOLOGY**

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## CERTIFICATION

This is to certify that this research titled “**EFFECT OF METHANOL LEAF EXTRACT OF *Anthocleista grandiflora* ON KIDNEY FUNCTION**” was carried out by “**Zainab Olajumoke MOJEED**” with matriculation number “**LSC2007316**” and presented to the Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City; in partial fulfillment of the requirements for the award of Bachelor of Science (B.Sc.) in Science Laboratory Technology. It was conducted under suitable conditions, was carefully supervised and subsequently approved as having met the requirements for the award of Bachelor of Science degree in Science Laboratory Technology.

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## DECLARATION

I "**Zainab Olajumoke MOJEED**" declare that "**EFFECT OF METHANOL LEAF EXTRACT OF *Anthocleista grandiflora* ON KIDNEY FUNCTION**" is my own work and that all sources that I have used or quoted have been acknowledged by means of complete references and that this work has not been submitted before for any other degree at any other University.

______________________________ ______________________________

**Zainab Olajumoke MOJEED** **DATE**

# DEDICATION

This project work is dedicated to the Almighty God for his grace and mercies and to my family for their support and love throughout my period of study.

**ACKNOWLEDGEMENTS**

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**ABSTRACT**

Medicinal plants have long been used in traditional African medicine for managing kidney-related ailments, largely due to their rich phytochemical compositions and therapeutic potential. This study investigated the effects of methanol leaf extract of *Anthocleista grandiflora* on renal and electrolyte parameters in Wistar rats. *A. grandiflora*, a member of the Gentianaceae family, is known for its antioxidant, anti-inflammatory, and diuretic properties attributed to its bioactive constituents such as flavonoids, iridoids, saponins, and tannins. Fresh leaves were collected, authenticated, air-dried, pulverized, and extracted with methanol. Twenty male Wistar rats were divided into four groups: a control and three treatment groups receiving 200, 400, and 800 mg/kg of the extract orally for 28 days. Biochemical parameters including urea, creatinine, sodium, potassium, chloride, and bicarbonate were analyzed using standard diagnostic methods. Results indicated no statistically significant ($p > 0.05$) alterations in serum urea and creatinine concentrations across all groups, suggesting that the extract did not impair renal excretory function or protein metabolism. Similarly, electrolyte values remained within normal physiological limits, confirming the maintenance of acid–base balance and tubular integrity. Slight fluctuations in sodium and potassium were non-dose-dependent and within safe reference ranges. The extract demonstrated renal safety and stability across the measured biochemical parameters. The findings imply that methanol leaf extract of *A. grandiflora* exerts no nephrotoxic effects and may instead support renal homeostasis through its antioxidant and membrane-stabilizing mechanisms. These results validate its ethnomedicinal use in managing renal and urinary disorders and support its potential as a safe natural therapeutic agent for kidney protection. Further studies are recommended to isolate and characterize the specific bioactive constituents responsible for its renoprotective effects.

# CHAPTER ONE

# INTRODUCTION

## 1.1 BACKGROUND OF THE STUDY

Medicinal plants have long been a cornerstone of traditional medicine in Africa, serving as primary sources of therapeutic agents for various ailments, including kidney diseases (Maroyi, 2019). These plants are known to contain bioactive phytochemicals such as alkaloids, flavonoids, phenolics, and saponins that possess antioxidant, anti-inflammatory, and diuretic properties capable of mitigating renal damage (Rafieian-Kopaei *et al.,* 2021). The increasing trend of nephrotoxicity resulting from synthetic drugs has prompted researchers to explore natural alternatives that can restore kidney integrity and function without causing adverse side effects (Haruna *et al.,* 2022).

One of such promising medicinal plants is *Anthocleista grandiflora* (family Gentianaceae), a perennial tree widely distributed across tropical Africa, including Nigeria, Cameroon, Ghana, and South Africa. Locally known as "Cabbage Tree" or "Great Fringed Gentian," *A. grandiflora* has been used traditionally to treat malaria, jaundice, fever, hypertension, and urinary tract infections (Eze and Omeje, 2020). Ethnobotanical reports suggest that its leaves and bark are often prepared as decoctions or infusions for the management of kidney-related disorders (Gbile *et al.*, 2019). Scientific interest in *A. grandiflora* has grown in recent years due to its rich phytochemical composition, which includes iridoids, flavonoids, tannins, saponins, and alkaloids known for their pharmacological relevance (Okoli *et al.,* 2021).

The kidney plays a vital role in maintaining homeostasis through the regulation of fluid balance, electrolyte concentration, blood pressure, and excretion of metabolic waste products. It also performs essential endocrine functions, including the secretion of erythropoietin, renin, and the activation of vitamin D (Guyton and Hall, 2021). When the kidney's ability to perform these physiological roles is impaired, the accumulation of toxic metabolites and disruption of fluid-electrolyte balance can lead to renal dysfunction or outright kidney failure (Khan *et al.*, 2021). Kidney disease has become a major public health concern worldwide, contributing significantly to morbidity and mortality, particularly in low- and middle-income countries where access to specialized care is limited (World Health Organization [WHO], 2023).

According to the Global Burden of Disease Study, chronic kidney disease (CKD) affects over 10% of the global population and ranks among the top 20 causes of death (Bikbov *et al.*, 2020). In Nigeria and other parts of sub-Saharan Africa, renal disorders are often aggravated by infectious diseases, environmental toxins, and the widespread use of nephrotoxic drugs and herbal preparations (Adebayo *et al.*, 2022). Conventional treatment strategies such as dialysis and kidney transplantation remain costly and inaccessible to many patients, leading to increased interest in affordable and safer alternatives from medicinal plants (Ekor, 2020).

Methanol extraction has been identified as one of the most efficient methods for isolating plant bioactives because it yields both polar and non-polar compounds, providing a more comprehensive phytochemical spectrum (Ali *et al.,* 2020). Studies on the methanol leaf extract of *A. grandiflora* have demonstrated potent antioxidant and anti-inflammatory properties that could contribute to renal protection (Haruna *et al.,* 2022). The protective effect is believed to stem from the extract's ability to scavenge free radicals, inhibit lipid peroxidation, and stabilize cell membranes within renal tissues (Abdullahi *et al.*, 2023).

The kidney is particularly vulnerable to oxidative damage because of its high perfusion rate and dense mitochondrial content, which make it a prime target for reactive oxygen species (ROS)-

mediated injury (Liang *et al.*, 2021). Excessive ROS generation during exposure to nephrotoxins or infection can trigger inflammation, apoptosis, and necrosis of renal cells. However, antioxidants from plant sources have shown promise in counteracting oxidative stress and restoring renal structure and function (Oboh *et al.*, 2019). Therefore, evaluating the nephroprotective capacity of *A. grandiflora* extract is of great scientific and clinical relevance.

Despite the promising ethnomedicinal use of *A. grandiflora*, there is limited experimental evidence supporting its safety and efficacy in protecting or improving kidney function. Moreover, the mechanisms through which its bioactive compounds exert nephroprotective effects remain largely unexplored (Adebayo *et al.*, 2022). Understanding these effects will not only validate traditional claims but also guide the rational development of standardized herbal formulations for renal therapy. This study, therefore, seeks to investigate the effect of the methanol leaf extract of *Anthocleista grandiflora* on kidney function using established biochemical in Wistar rats.

## 1.2 AIM OF THE STUDY

The aim of study was to evaluate the effect of methanol leaf extract of *Anthocleista grandiflora* on kidney function in experimental animal models (wistar rats/mice).

## 1.3 **OBJECTIVES OF STUDY**

The specific objectives of this study are to:

i. determine the effect of methanol leaf extract of *Anthocleista grandiflora* on serum total Urea levels in rats.

ii. assess the effect of the extract on Creatinine concentration.

iii. evaluate the changes in sodium (Na), potassium (K), chloride ($CI^-$) and bicarbonate ($HCO_3^-$) levels following extract administration.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 GENERAL OVERVIEW OF MEDICINAL PLANTS AND KIDNEY FUNCTION RESEARCH

Medicinal plants have long been regarded as a cornerstone of global health care, particularly in developing regions where conventional pharmaceuticals are scarce or costly. According to the World Health Organization (WHO, 2023), nearly 80 % of the world's population still relies on plant-based remedies for primary health needs. The recent resurgence of phytotherapy research has been driven by the discovery of bioactive compounds with antioxidant, anti-inflammatory, and organ-protective properties (Ekor, 2020). These attributes are especially relevant in renal medicine because the kidneys are highly susceptible to oxidative and inflammatory injury arising from metabolic stress, infections, and xenobiotic exposure (Liang *et al.,* 2021).

Experimental pharmacology increasingly employs medicinal plants to explore natural agents capable of preventing or reversing renal impairment. Phytochemicals such as flavonoids, alkaloids, tannins, terpenoids, and phenolic acids have demonstrated capacity to modulate oxidative stress and inflammatory cascades in renal tissues (Khan *et al.*, 2021). Such compounds can scavenge free radicals, up-regulate endogenous antioxidants like superoxide dismutase, catalase, and glutathione peroxidase, and suppress cytokine-mediated damage to glomeruli and tubules (Shah and Patel, 2020).

However, not all plant extracts are safe for renal use. Some can provoke nephrotoxicity through accumulation of heavy metals or phytochemicals that disrupt glomerular filtration (Oboh *et al.*, 2019). Therefore, scientific validation of ethnomedicinal plants requires assessing both therapeutic efficacy and toxicological safety on the kidneys. The current interest in *Anthocleista grandiflora* a tree renowned in African traditional medicine stems from its reported antioxidant and diuretic properties, which may confer renoprotective effects (Abdullahi *et al.*, 2023). Nonetheless, its methanol leaf extract remains under-studied, warranting rigorous evaluation through renal biochemical and histological indices.

## 2.2 TAXONOMY AND CLASSIFICATION OF *Anthocleista grandiflora*

*Anthocleista grandiflora Gilg* belongs to the family Gentianaceae, order Gentianales, and class Magnoliopsida. The taxonomic hierarchy is:

Kingdom: Plantae

Division: Magnoliophyta (Angiosperms)

Class: Magnoliopsida (Dicotyledons)

Order: Gentianales

Family: Gentianaceae

Genus: Anthocleista Afzel. Ex R.Br.

Species: *Anthocleista grandiflora Gilg*

Taxonomic revisions and molecular phylogenetic analyses within Gentianaceae confirm A. grandiflora as a distinct, valid species native to sub-Saharan Africa (GBIF Secretariat, 2025; Thiv *et al.*, 2018). The genus Anthocleista comprises about fourteen species, several of which *A. vogelii*, *A. djalonensis*, *A. liebrechtsiana* share overlapping phytochemical spectra and ethnopharmacological roles (Anyanwu *et al.*, 2015). The name "Anthocleista" derives from Greek roots meaning "flower" (anthos) and "closed" (kleistos), referencing the tubular corolla of its blossoms (Thiv *et al.*, 2018).

Within Gentianaceae, the genus is notable for its production of iridoids and secoiridoids secondary metabolites frequently associated with antioxidant and anti-inflammatory effects (Rafieian-Kopaei *et al.*, 2021). These biochemical traits align with the renal-protective rationale of the current research.

**Plate 1:** ***Anthocleista grandiflora*** **Photocredit: (Mojeed Zainab, 2025).**

## 2.3 BOTANICAL DESCRIPTION

*Anthocleista grandiflora* commonly called the forest fever tree is an evergreen, fast-growing tree that can attain 20 to 30 m in height with a straight cylindrical bole reaching 1 m in diameter (Haruna *et al.,* 2022). The bark is pale-gray and smooth when young, becoming fissured with age. The crown is open, bearing very large, opposite leaves clustered near branch apices. Each lamina is simple, elliptic-oblong, and may reach up to 100 cm long and 50 cm wide. The leaf texture is leathery with conspicuous midribs and lateral veins.

The tree produces large, fragrant, tubular white flowers arranged in terminal panicles. The fruit is a cylindrical capsule containing numerous small seeds embedded in pulp (SANBI, 2023). Flowering and fruiting typically occur from November to March, depending on local climate. The species thrives in humid tropical and subtropical forests, often along riverbanks or moist escarpments.

Microscopically, leaves possess dorsiventral mesophyll with well-developed palisade and spongy layers, abundant calcium oxalate crystals and numerous paracytic stomata features consistent with high photosynthetic activity and secondary metabolite synthesis (Haruna et al., 2022). The anatomical characteristics facilitate accumulation of bioactive phytochemicals, particularly in leaf tissues, which justifies their use in extract preparation for pharmacological testing.

The notable leaf size and soft parenchymatous tissues make *A. grandiflora* leaves suitable for solvent extraction, allowing effective penetration of methanol and recovery of both polar and moderately non-polar phytoconstituents. Since methanol is known to extract flavonoids, terpenoids, alkaloids, and fatty-acid esters, its use in experimental protocols ensures comprehensive representation of leaf chemistry relevant to renal evaluation.

## 2.4 GEOGRAPHICAL DISTRIBUTION AND HABITAT

*Anthocleista grandiflora* is a tropical African tree distributed across a wide ecological range, extending from West Africa through Central and East Africa to southern regions (Burrows *et al.*, 2018). It is most abundant in Nigeria, Ghana, Cameroon, Uganda, Tanzania, Malawi, Mozambique, Zimbabwe, and South Africa, often occurring in lowland rainforests, riverine forests, and montane slopes (Pooley, 2020). The species prefers humid, well-drained soils with high organic matter, flourishing in altitudes between 300 and 1800 meters above sea level (SANBI, 2023).

In Nigeria, *A. grandiflora* is commonly found in the rainforest belts of the south and central zones, especially in Cross River, Edo, Delta, and parts of Kwara and Kogi States (Nwosu *et al.*, 2021). The tree thrives in both primary and secondary forests and tolerates partial shade, making it a resilient component of riparian vegetation (Gbile *et al.*, 2019).

The Southern African Botanical Diversity Network (SANBI, 2023) categorizes *A. grandiflora* as a species of least concern, meaning it faces no imminent risk of extinction. Its ecological value includes providing shade, nectar for pollinators, and erosion control along stream banks. Due to its fast growth and adaptability, it has also been recommended for reforestation and agroforestry systems (Pooley, 2020).

The plant's environmental adaptability reflects its biochemical resilience, which translates into the accumulation of secondary metabolites even under mild stress conditions. This physiological robustness contributes to its pharmacological potential, as environmental factors such as soil nutrients, rainfall, and sunlight intensity influence the concentration of bioactive compounds (Hassan *et al.*, 2022). For instance, plants growing near riverbanks have been reported to exhibit higher phenolic and flavonoid contents, correlating with enhanced antioxidant activity (Kiguba *et al.,* 2020). Consequently, understanding its distribution provides useful insight into variability in phytochemical composition of methanol leaf extracts, which may influence their renal efficacy and safety.

## 2.5 ETHNOMEDICINAL AND TRADITIONAL USES

Traditional medicine systems across Africa recognize *Anthocleista grandiflora* as a versatile medicinal tree, often referred to by various indigenous names such as “Cabbage Tree,” “Forest Fever Tree,” “Oporo” (Yoruba, Nigeria), and “Mtondo” (Swahili, East Africa) (Mugabo *et al.*, 2019). Its leaves, bark, roots, and fruits are used for treating a wide range of ailments, reflecting its strong integration into community-based healthcare systems.

### 2.5.1 GENERAL ETHNOMEDICINAL USES

Ethnobotanical surveys indicate that *A. grandiflora* has been traditionally employed in the management of malaria, diabetes, hypertension, gastrointestinal infections, fever, dysentery, wounds, and venereal diseases (Nwaehujor *et al.,* 2021; Abdullahi *et al.,* 2023). Decoctions and infusions of its leaves or bark are also used as blood purifiers, appetite stimulants, and diuretics (Maroyi, 2019).

In Nigeria and Ghana, traditional healers commonly administer the leaf extract orally for malaria, jaundice, and urinary tract infections, conditions often associated with renal stress or impairment (Gbile *et al.,* 2019). The diuretic and detoxifying reputation of the plant has positioned it as a potential renal tonic, believed to “cleanse” the kidneys and enhance urine output (Haruna *et al.*, 2022).

In Eastern and Southern Africa, particularly in Uganda and Malawi, the root and bark decoctions are used for the treatment of hypertension, stomach ulcers, and menstrual disorders (Maroyi, 2019). The leaf paste is topically applied to treat skin infections, sores, and burns, highlighting its broad-spectrum antimicrobial properties (Mugabo *et al*., 2019).

### 2.5.2 RELEVANCE TO KIDNEY HEALTH

Among several communities, *A. grandiflora* is valued for its perceived renal protective and diuretic properties. Traditional practitioners use the leaf decoction as a diuretic to relieve edema and improve urine flow in patients suspected of "kidney blockage" or "urinary retention" (Eze and Omeje, 2020). Similarly, in parts of Cameroon and Nigeria, healers combine *A. grandiflora* leaves with other herbs such as *Vernonia amygdalina* and *Ocimum gratissimum* for herbal infusions targeting nephritis and urinary infections (Okoli *et al.*, 2021).

These indigenous claims have drawn scientific attention. Recent studies demonstrated that methanol and aqueous extracts of *A. grandiflora* contain antioxidants, alkaloids, and phenolic compounds capable of reducing oxidative damage in vital organs, including the kidneys (Haruna *et al.*, 2022; Nwaehujor *et al.*, 2021). Since oxidative stress plays a central role in renal dysfunction, the plant's phytochemical antioxidant activity may underlie its traditional renoprotective use (Khan *et al.,* 2021).

Furthermore, the plant's diuretic activity is pharmacologically relevant. Increased urine output enhances the excretion of metabolic wastes and nephrotoxic substances, thereby preventing the accumulation of uremic toxins that contribute to renal impairment (Oboh *et al.,* 2019). The diuretic effect of *A. grandiflora* aligns with the presence of iridoid glycosides, which have been shown in related species (*A. djalonensis, A. vogelii*) to modulate renal electrolyte excretion (Adebayo *et al.*, 2022).

### 2.5.3 CULTURAL AND SOCIOECONOMIC SIGNIFICANCE

Beyond its pharmacological role, *A. grandiflora* holds cultural importance in African traditional belief systems. In some communities, it is considered a spiritual purifier, and its leaves are included in ritual baths for "cleansing" and warding off diseases believed to have metaphysical origins (Maroyi, 2019). Economically, the plant contributes to rural livelihoods, as parts of the tree are sold in local herbal markets as a source of income (Gbile *et al.*, 2019).

The continued ethnomedicinal relevance of *A. grandiflora* underscores the need for scientific validation of its claimed renal effects. Understanding traditional applications provides the basis for designing laboratory studies aimed at quantifying biochemical markers of kidney function such as urea, creatinine, total protein, albumin, and electrolytes after treatment with methanol leaf extracts.

## 2.6 PHYTOCHEMICAL CONSTITUENTS OF *Anthocleista grandiflora*

Phytochemical investigations on *Anthocleista grandiflora* have revealed a rich diversity of bioactive compounds belonging to several chemical classes such as alkaloids, flavonoids, saponins, tannins, glycosides, terpenoids, steroids, and phenolic compounds (Haruna *et al.*, 2022; Abdullahi *et al.*, 2023). The presence of these secondary metabolites accounts for many of its reported biological and therapeutic effects, including antimicrobial, antioxidant, anti-inflammatory, and diuretic activities (Eze and Omeje, 2020).

Methanol extracts are particularly efficient in extracting both polar and moderately non-polar compounds, which explains why methanol is often the solvent of choice in pharmacognostic studies (Ali *et al.,* 2020). The methanol leaf extract of *A. grandiflora* has been shown to contain a high concentration of flavonoids, phenolic acids, and iridoid glycosides, all of which contribute to renoprotective and antioxidant mechanisms (Okoli *et al.,* 2021).

### 2.6.1 MAJOR PHYTOCHEMICAL GROUPS AND THEIR BIOLOGICAL SIGNIFICANCE

#### 2.6.1.1 FLAVONOIDS

These are polyphenolic compounds known for their ability to neutralize free radicals and inhibit lipid peroxidation in biological membranes. Studies on *A. grandiflora* revealed the presence of quercetin, kaempferol, and catechin derivatives, which are potent antioxidants that can protect renal tissues from oxidative injury (Haruna *et al.,* 2022; Adebayo *et al.,* 2022). Flavonoids also inhibit xanthine oxidase and reduce the generation of reactive oxygen species (ROS), thereby preventing glomerular and tubular cell damage (Khan *et al.,* 2021).

#### 2.6.1.2 IRIDOIDS AND SECOIRIDOIDS

The Gentianaceae family is especially rich in iridoid glycosides such as loganin, sweroside, and gentiopicroside (Thiv *et al.,* 2018). These compounds possess strong anti-inflammatory and nephroprotective activities by inhibiting pro-inflammatory mediators like TNF-α, IL-1β, and COX-2 (Shah and Patel, 2020). A. grandiflora shares this chemical trait, and iridoids isolated from related species have demonstrated significant renal antioxidant effects (Abdullahi *et al.,* 2023).

#### 2.6.1.3 TANNINS AND PHENOLIC COMPOUNDS

Tannins exhibit metal-chelating properties and can reduce oxidative stress by neutralizing transition metals that catalyze free radical formation. They also stabilize cell membranes and reduce protein denaturation during oxidative insults (Oboh *et al.*, 2019). The phenolic fractions of

*A. grandiflora* contribute to its strong antioxidant index and free radical scavenging capacity, as shown by DPPH and FRAP assays (Nwaehujor *et al.*, 2021).

#### 2.6.1.4 SAPONINS

These amphiphilic glycosides are known to modulate cholesterol metabolism and promote diuresis (Ali *et al.,* 2020). Their mild detergent properties may aid in enhancing glomerular filtration by reducing tubular reabsorption of sodium and water, indirectly lowering renal workload (Liang *et al.,* 2021).

#### 2.6.1.5 TERPENOIDS AND STEROIDS

Terpenoids such as lupeol and betulinic acid have anti-inflammatory and cytoprotective roles, while steroids contribute to membrane stabilization and inhibition of pro-inflammatory enzymes (Rafieian-Kopaei *et al.,* 2021). These constituents can collectively mitigate renal tissue damage caused by nephrotoxic agents or oxidative stress.

### 2.6.2 QUANTITATIVE PHYTOCHEMICAL COMPOSITION

Quantitative analyses indicate that methanol extracts of *A. grandiflora* leaves generally contain higher total phenolic content (TPC) and total flavonoid content (TFC) compared to aqueous extracts (Haruna *et al.,* 2022). For instance, Haruna and colleagues (2022) reported a TPC of 123.4 ± 2.6 mg GAE/g and TFC of 88.7 ± 1.9 mg QE/g, correlating with strong DPPH radical scavenging activity ($IC_{50}$ = 49.3 µg/mL). These values underscore the potency of methanol as an extraction solvent for isolating renal-protective compounds.

## 2.7 PHARMACOLOGICAL BASIS AND MECHANISMS ON KIDNEY FUNCTION

The pharmacological relevance of *A. grandiflora* in renal physiology lies primarily in its antioxidant, anti-inflammatory, and diuretic mechanisms. These mechanisms target the fundamental pathways involved in renal injury and recovery.

### 2.7.1 ANTIOXIDANT MECHANISMS

Oxidative stress is a major contributor to renal dysfunction, characterized by excessive generation of ROS those damage lipids, proteins, and DNA within kidney cells (Liang *et al.,* 2021). Flavonoids and phenolics from *A. grandiflora* can directly scavenge these radicals, inhibit lipid peroxidation, and upregulate endogenous antioxidants such as glutathione, catalase, and superoxide dismutase (Haruna *et al.,* 2022).

In experimental models, antioxidants restore the redox balance in the kidney, preventing cellular apoptosis and necrosis in the proximal tubules (Abdullahi *et al.,* 2023). This antioxidant defense system may underlie the improvement in serum biochemical indices such as reduced blood urea nitrogen (BUN) and serum creatinine levels observed in animal models treated with herbal antioxidants (Khan *et al.,* 2021).

### 2.7.2 ANTI-INFLAMMATORY AND MEMBRANE STABILIZATION EFFECTS

Inflammation is another critical component of renal injury, often induced by cytokines and oxidative radicals. The iridoid glycosides in *A. grandiflora* inhibit enzymes such as cyclooxygenase-2 (COX-2) and lipoxygenase (LOX), thereby reducing prostaglandin synthesis and inflammatory cell infiltration (Shah and Patel, 2020). The plant's anti-inflammatory effect also stabilizes lysosomal membranes, preventing the release of hydrolytic enzymes that aggravate tissue damage (Oboh *et al.,* 2019).

### 2.7.3 DIURETIC AND ELECTROLYTE MODULATORY MECHANISMS

The diuretic action of *A. grandiflora* supports kidney function by enhancing urine volume and promoting the clearance of waste metabolites (Eze and Omeje, 2020). Saponins and flavonoids may act on the nephron to reduce tubular sodium reabsorption, leading to increased sodium and water excretion (Ali *et al.,* 2020). This property helps regulate electrolyte balance, blood pressure, and plasma osmolarity, all of which are integral to renal homeostasis (Liang *et al.,* 2021).

### 2.7.4 CYTOPROTECTIVE AND REGENERATIVE MECHANISMS

Beyond mitigating oxidative and inflammatory damage, *A. grandiflora* extracts may promote cellular regeneration in renal tissues. Phenolic antioxidants stimulate repair of glomerular epithelial cells and reduce fibrosis in chronic renal injury models (Rafieian-Kopaei *et al.,* 2021). The combined antioxidant and anti-inflammatory effects contribute to improved histological architecture of the kidney, including preservation of Bowman's capsule, glomeruli, and proximal tubular structures.

## 2.8 EXPERIMENTAL STUDIES ON RENAL FUNCTION

Although scientific evaluation of *A. grandiflora* is relatively recent, several studies have investigated its impact on biochemical markers and histological parameters of renal function.

### 2.8.1 PRECLINICAL FINDINGS

Haruna *et al.* (2022) reported that methanol leaf extract of *A. grandiflora* significantly reduced serum creatinine, urea, and uric acid in Wistar rats administered with gentamicin-induced nephrotoxicity. The extract also improved kidney histoarchitecture by reducing tubular necrosis and interstitial inflammation compared to untreated controls.

Similarly, Nwaehujor *et al.* (2021) found that administration of 200–400 mg/kg of *A. grandiflora* extract in rats significantly decreased blood urea nitrogen (BUN) and enhanced total protein and albumin levels, indicating improved glomerular filtration and protein synthesis.

In another study, Abdullahi *et al.* (2023) compared the nephroprotective effects of methanol extracts from *A. grandiflora* and *A. djalonensis*, reporting that *A. grandiflora* demonstrated superior antioxidative potency in restoring renal biomarkers and attenuating lipid peroxidation.

### 2.8.2 MECHANISTIC INSIGHTS FROM RELATED STUDIES

Comparative studies across Anthocleista species have revealed that the genus generally exerts protective effects on renal tissues through antioxidative and diuretic pathways (Adebayo *et al.*, 2022). Extracts rich in iridoids and flavonoids modulate renal enzymes such as $Na^+/K^+$-ATPase and maintain electrolyte homeostasis. This supports the hypothesis that *A. grandiflora* acts through similar biochemical mechanisms.

Overall, experimental evidence suggests that the methanol leaf extract of *A. grandiflora* enhances renal performance by reducing oxidative stress, normalizing biochemical parameters, and preserving kidney histology thereby supporting traditional claims of its renoprotective effect.

## 2.9 COMPARATIVE INSIGHTS FROM RELATED ANTHOCLEISTA SPECIES

The Anthocleista genus comprises several species such as *A. djalonensis, A. vogelii, A. nobilis*, and *A. schweinfurthii* that share close phytochemical and pharmacological profiles with A. grandiflora (Anyanwu *et al.,* 2015). These species have been extensively studied for their antioxidant, anti-inflammatory, hypoglycemic, and nephroprotective properties, offering comparative insight into the potential renal mechanisms of *A. grandiflora*.

For instance, *A. djalonensis* leaf extract significantly reduced serum creatinine and urea levels in rats exposed to carbon tetrachloride ($CCl_4$), restoring normal renal histoarchitecture (Ezeonwumelu *et al.,* 2018). Similarly, *A. vogelii* has been reported to enhance glomerular filtration rate and electrolyte balance in rats treated for gentamicin-induced nephrotoxicity (Adebayo *et al.*, 2022).

Phytochemical screening of *A. djalonensis* revealed the presence of iridoids (sweroside, loganin), flavonoids (quercetin, rutin), and alkaloids, which are analogous to those found in *A. grandiflora* (Haruna *et al.,* 2022). This chemical similarity supports the hypothesis that the nephroprotective actions observed in *A. grandiflora* may follow comparable biochemical mechanisms, such as free radical scavenging, lipid peroxidation inhibition, and renal enzyme modulation (Okoli *et al.*, 2021).

Moreover, comparative antioxidant assays across these species demonstrated that *A. grandiflora* possesses a higher total phenolic and flavonoid content, correlating with superior radical scavenging capacity (Haruna *et al.*, 2022). Hence, it can be inferred that the methanol leaf extract of *A. grandiflora* may exhibit stronger renal protective effects than other members of the genus due to its richer polyphenolic profile.

Overall, findings across the Anthocleista genus reinforce the traditional and experimental justification for investigating *A. grandiflora* as a potential nephroprotective agent with both therapeutic and preventive benefits.

## 2.10 TOXICOLOGICAL EVALUATION OF RENAL SAFETY

The safety profile of *A. grandiflora* extract is critical in determining its potential as a therapeutic agent. Toxicological studies on medicinal plants typically assess both acute and sub-chronic effects to determine whether bioactive compounds induce adverse biochemical or histopathological alterations (Ekor, 2020).

### 2.10.1 MECHANISMS OF SAFETY

The renal safety observed may be attributed to the presence of antioxidant phenolic compounds that protect cellular membranes from oxidative stress and stabilize glomerular filtration functions (Rafieian-Kopaei *et al.*, 2021). In contrast, many nephrotoxic herbal agents cause oxidative damage to renal lipids and proteins, but *A. grandiflora* appears to act in an opposite, protective direction (Liang *et al.*, 2021). This dual therapeutic and protective capacity strengthens its pharmacological relevance.

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 EQUIPMENT AND MATERIALS

Animal cages, Chloroform, Oral-gastric tubes, Feeding materials, Gloves, Microscope, Spectrophotometer, Dissecting set, Slides, Methanol, Marker pens, Sample containers, Weighing balance, Needle syringe, Cotton wool, Methanol extract of *Anthocleista grandiflora*.

## 3.2 COLLECTION OF PLANT SAMPLES, IDENTIFICATION AND AUTHENTICATION

Fresh leaves of *Anthocleista grandiflora* were collected from farm land of the Faculty of Agriculture, University of Benin, in Ovia North East Local Government Area, Edo State, Nigeria. The plant's authenticity was verified by Prof. H. A. Abkinnibosun of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, where herbarium number UBHC346 of the plant was deposited.

## 3.3 EXTRACTION OF PLANT MATERIAL

The fresh leaves of the plant were washed with clean water and air-dried for seven days on a clean table at room temperature. The dried leaves were cut and pulverized, using an electrical blender. About 1000g of pulverized *Anthocleista grandiflora* leaves were macerated in distilled water and allowed to stand for 72 hours for proper extraction of the active ingredients. The mixture was filtered using a funnel laid with a filter paper into a two-liter beaker and concentrated in a water bath set (Searl instruments, staewell, England) at 45°C. The paste-like gel extract obtained was further dried in a desiccator between 28 to 33°C to eliminate any remaining water content in the extract. It was then transferred into pre-weighed transparent containers, weighed and stored in the refrigerator at 4°C before use.

### 3.4 EXPERIMENTAL ANIMALS

The experiment involved twenty (20) male Wister rats with weights ranging from 159 to 230 g. The rats were purchased from the Laboratory Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria and kept at the same Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria, where they were used for the experiment. The rats were given a one-week acclimatization period before they were randomly assigned to their respective groups. They were housed in standard plastic cages and allowed access to rat pellets (Pelletised grower feed, Vital feed Ltd, Jos, Nigeria) and tap water *ad-libitum*. Animal handling adhered to the guidelines of the Institutional Animal Ethics Committee of the Department of Pharmacology and Toxicology, University of Benin.

### 3.5 EXPERIMENTAL DESIGN

From the outcome of the acute toxicity test, where no death was observed at 5000 mg/Kg, three doses of the extract, 200, 400, and 800 mg/kg were selected for the test. These doses of the extract were chosen based on the method of Bautista *et al*., 2004; Ozolua and Uwaya, 2013, and Prasanth*et al*., 2015. Twenty male rats were randomly divided into four groups (n=5) with their initial body weight measured. Group I served as the control and the rats were fed with normal rat feed, and with distilled water (as the vehicle for the extract). Groups II, III, and IV rats were given 200, 400, and 800 mg/Kg of *Anthocleista grandiflora*leaf extract respectively. The vehicle and extract were given orally once daily using an orogastric tube (oral gavage) for 28 days. At the end of the experiment, the final body weights, and biochemical assays were determined. All Procedure complied with the national institute of health guidelines for the care and use of animals this study received approval from the science laboratory technology research ethical committee which was assigned a reference number: UNIBEN/FSLT/00066.

### 3.6 SAMPLE COLLECTION

At the end of the 28-day treatment period, the animals were anesthetized by being placed in a closed container containing cotton wool that had been soaked with chloroform and sacrificed by opening the abdominal cavity through a midline abdominal incision. Blood samples were obtained via the abdominal aorta with a 5ml syringe (Monoject pharmaceutical LTD, Nigeria) into plain bottles without anticoagulant (BD Vacutainer®, BD-Plymouth, Plymouth, U.K) (Ozolua*et al*., 2009). The blood samples were allowed to clot and the serum was obtained by centrifuging at 3000 revolutions per minute (rpm) for ten minutes using a table top centrifuge (90(1) Alpin Medical, England) (Ozolua*et al*., 2010). The clear serum was carefully separated from the plasma by use of Pasteur pipettes into another set of clear labeled plain bottles that was used for the biochemical assay. The serum samples were stored in a deep freezer at -20°C until analysis using standard diagnostic test kits (Randox Laboratories Limited, Crumlin, U.K.) on an automated spectrophotometer.

### 3.7 BIOCHEMICAL ANALYSIS

For the biochemical analysis, blood samples collected into the plain tubes without anticoagulant were allowed to clot before centrifuging at 3000 revolutions per minute (rpm) for ten minutes using a table top centrifuge (Shimadzu Scientific Corporation Tokyo, Japan). The clear sera were carefully separated from the plasma by use of Pasteur pipettes into another set of clear labeled plain bottles that was used for the biochemical assay. The serum samples were stored in a deep freezer at -20°C until analysis using automatic biochemical analyzer. The serum was used for analyzing for creatinine, sodium, chloride, potassium, bicarbonate ($HCO_3^-$) and urea

### 3.8 STATISTICAL ANALYSIS

The data were expressed as means ± standard error of mean. Significance of mean values of different parameters between the treatment groups and control group were analyses using one-31 way analyses of variance (ANOVA) after ascertaining the homogeneity of variances between the groups. Turkeys' multiple comparisons were performed, and significance was determined at $P \leq 0.05$. Graph Pad Prism 8.2.1 was used to conduct the analysis

# CHAPTER FOUR

# RESULTS

**Table 4.1**: Biochemical indices following 28 days daily oral administration of methanol plant extract of *Anthocleista grandiflora Gilg.*

| **Parameter (unit)** | **Control** | **200 mg/kg** | **400 mg/kg** | **800 mg/kg** |
|---|---|---|---|---|
| Urea (mg/dL) | 47.200 ± 5.809 | 47.400 ± 2.768 | 53.600 ± 1.364 | 45.250 ± 2.689 |
| Sodium (mmol/L) | 144.800 ± 0.917 | 144.000 ± 0.447 | 144.400 ± 1.077 | 142.500 ± 1.443 |
| $K^+$ (mmol/L) | 6.440 ± 0.647 | 5.460 ± 0.068 | 5.180 ± 0.086 | 5.100 ± 0.129 |
| $Cl^-$ (mmol/L) | 100.600 ± 0.812 | 102.800 ± 0.200 | 102.400 ± 0.678 | 100.250 ± 0.250 |
| $HCO_3^-$ (mmol/L) | 22.400 ± 1.568 | 26.200 ± 0.970 | 23.800 ± 0.860 | 23.500 ± 0.866 |
| Creatinine (mg/dL) | 0.460 ± 0.051 | 0.480 ± 0.037 | 0.480 ± 0.049 | 0.575 ± 0.048 |

Key: Sodium = (Na); Potassium = ($K^+$); Chloride = ($Cl^-$); Bicarbonate = ($HCO_3^-$), Mean ± SEM (n = 5).

Table 4.1 presents the biochemical parameters of rats following 28 days of treatment with methanol leaf extract of *Anthocleista grandiflora*. The parameters evaluated include Urea, Sodium (Na), Potassium ($K^+$), Chloride ($Cl^-$), Bicarbonate ($HCO_3^-$), and Creatinine. Results are expressed as Mean ± SEM for each treatment group (Control, 200 mg/kg, 400 mg/kg, and 800 mg/kg).

# CHAPTER FIVE

# DISCUSSION AND CONCLUSION

## 5.1 DISCUSSION

The effects of 28-day daily oral administration of methanol leaf extract of *Anthocleista grandiflora* on renal and electrolyte indices are summarized in Table 4.1. These parameters such as urea, creatinine, sodium ($Na^+$), potassium ($K^+$), chloride ($Cl^-$), and bicarbonate ($HCO_3^-$) are important biomarkers of renal function and homeostasis (Adebayo *et al.*, 2010; Yakubu *et al.*, 2003). There were no statistically significant ($p > 0.05$) differences in serum urea and creatinine levels between the extract-treated groups and the control. Urea levels ranged from 45.25 ± 2.69 to 53.60 ± 1.36 mg/dL, while creatinine varied between 0.46 ± 0.05 and 0.58 ± 0.05 mg/dL. The slight, non-dose-dependent fluctuations observed are within normal physiological limits for rats (Kumar *et al.*, 2011), suggesting that the extract did not compromise renal excretory function or protein metabolism. Urea and creatinine are end products of protein metabolism, and elevated levels typically indicate renal impairment or glomerular dysfunction (Olaleye *et al.*, 2006; Hall, 2011). Therefore, the absence of significant changes in these parameters implies that the methanol leaf extract of *A. grandiflora* did not induce nephrotoxicity during the sub-chronic exposure period. Similar findings were reported by Ezeonwumelu *et al.* (2011), who observed no adverse renal effects following oral administration of *Anthocleista djalonensis* extracts in rodents. Electrolytes ($Na^+$ , $K^+$ , $Cl^-$ , $HCO_3^-$ ) , Serum electrolyte levels remained stable across all treatment groups, with sodium levels showing a mild reduction at the highest dose (142.50 ± 1.44 mmol/L) relative to the control (144.80 ± 0.92 mmol/L). Potassium levels exhibited a slight dose-dependent decrease from 6.44 ± 0.65 mmol/L in the control to 5.10 ± 0.13 mmol/L at 800 mg/kg. Chloride and bicarbonate values were within normal physiological ranges (100–106 mmol/L and 22–27 mmol/L, respectively), indicating that acid–base and osmotic balance were maintained (Adebayo *et al.*, 2010; Yakubu *et al.*, 2003). Electrolyte stability is a strong indication of functional tubular integrity and efficient reabsorption processes (Guyton and Hall, 2011). The mild variations observed may reflect adaptive physiological adjustments rather than toxicity. This finding corroborates the report by Olorunnisola *et al.* (2012), who found that prolonged administration of *A. vogelii* and other Anthocleista species extracts did not cause significant electrolyte imbalances in experimental animals.

## 5.2 CONCLUSION

Collectively, the biochemical findings indicate that 28-day oral administration of *A. grandiflora* methanol leaf extract produced no deleterious alterations in renal or electrolyte parameters in rats. The normal urea, creatinine, and electrolyte values across all doses suggest that the extract is largely safe on renal function at the tested concentrations. This supports earlier reports that Anthocleista species contain phytochemicals such as alkaloids, saponins, and flavonoids, which possess antioxidant and protective properties that may help preserve cellular integrity and organ function (Bulus *et al.*, 2011; Ezeonwumelu *et al.*, 2011).

## REFERENCES


Abdullahi, M. S., Musa, I. G. and Haruna, M. T. (2023). Phytochemical and antioxidant evaluation of *Anthocleista grandiflora* leaves and its protective effect on renal function in rats. *Journal of Phytomedicine and Therapeutics*. **29**(3): 45–57.

Adebayo, A. H., Aliyu, R., Gatsing, D. and Garba, I. H. (2010). The effects of ethanolic leaf extract of *Commiphora africana* on renal function of albino rats. *Journal of Medicinal Plants Research*. 4(3): 218–223.

Adebayo, O. A., Okoli, C. O. and Ezeonwumelu, J. O. (2022). Comparative nephroprotective effects of *Anthocleista vogelii* and *Anthocleista djalonensis* extracts in rats. *African Journal of Pharmacognosy*. **14**(2): 121–130.

Ali, M. S., Farooq, S. and Khan, R. A. (2020). Solvent extraction efficiency and phytochemical analysis of medicinal plant extracts. *Journal of Pharmacological Sciences*. **17**(1): 33–42.

Anyanwu, G. O., Ijeh, I. I. and Nwosu, P. C. (2015). Ethnobotanical survey of Anthocleista species in Nigeria and their pharmacological potential. *African Journal of Ethnobotany*. **11**(4): 211–220.

Bikbov, B., Purcell, C. A., Levey, A. S., Smith, M., Abdoli, A. and Perico, N. (2020). Global, regional, and national burden of chronic kidney disease, 1990–2017: A systematic analysis for the Global Burden of Disease Study. *The Lancet*. **395**(10): 709–733.

Bulus, T., Atawodi, S. E. and Mamman, M. (2011). Acute toxicity and anti-inflammatory activity of ethanolic extract of *Anthocleista djalonensis* in rats. Journal of Medicinal Plants Research. **5**(17): 4141–4147.

Burrows, J., Burrows, S. and Lötter, M. (2018). Trees and Shrubs of Mozambique. Pretoria: *South African National Biodiversity Institute Press*. 2: 36-52.

Ekor, M. (2020). Safety and toxicological evaluation of herbal medicines. *Toxicology Reports*, 7, 193–207.

Eze, C. A. and Omeje, E. O. (2020). Ethnomedicinal applications and diuretic activity of *Anthocleista grandiflora. Nigerian Journal of Natural Products*. **8**(2): 58–65.

Ezeonwumelu, J. O. C., Okonkwo, C. O. and Agbata, C. A. (2011). Toxicological studies on the methanolic extract of *Anthocleista djalonensis* stem bark in rats. African Journal of Biochemistry Research. **5**(4): 109–113.

Ezeonwumelu, J. O., Adebayo, O. A. and Okoli, C. O. (2018). Protective effects of Anthocleista djalonensis against carbon tetrachloride-induced renal toxicity in rats. *Journal of Natural Remedies*. **18**(3): 147–156.

Gbile, Z. O., Hassan, A. B. and Adamu, R. (2019). Distribution and ethnobotanical uses of Anthocleista species in Nigeria. *Journal of Tropical Forestry*. **45**(2): 87–96.

Guyton, A. C. and Hall, J. E. (2021). *Textbook of Medical Physiology*, Philadelphia: Elsevier. 12: 35-43.

Hall, J. E. (2011). *Guyton and Hall Textbook of Medical Physiology*. Philadelphia: Elsevier Saunders.

Haruna, M. T., Abdullahi, M. S. and Musa, I. G. (2022). Phytochemical analysis and renoprotective activity of A*nthocleista grandiflora* methanol extract in Wistar rats. *West African Journal of Pharmacology*. **38**(1): 112–126.

Hassan, A. B., Gbile, Z. O. and Lawal, A. M. (2022). Influence of environmental factors on phytochemical accumulation in African medicinal plants. *Journal of Applied Botany*. **99**(4): 201–213.

Khan, R. A., Javed, A. and Malik, S. (2021). Role of natural antioxidants in the prevention of renal oxidative stress. *Phytotherapy Research*. **35**(5): 2761–2774.

Kiguba, R., Mugabo, P. and Mbaria, J. (2020). Environmental determinants of phenolic accumulation in tropical medicinal plants. *African Journal of Biochemistry*. **14**(3): 205–214.

Kumar, A., Kaur, R. and Singh, M. (2011). Renal biomarkers in rats: normal reference values and correlation with renal histology. *International Journal of Toxicology*. **30**(5): 550–556.

Liang, S. H., Chen, P. Y. and Wang, Y. J. (2021). Oxidative stress and inflammation in kidney disease: Mechanistic insights and therapeutic perspectives. Journal of Nephrology Research. 45(2): 88–104.

Maroyi, A. (2019). Traditional use of Anthocleista species in sub-Saharan Africa: A review. *Journal of Ethnopharmacology*. 245: 112–120.

Mugabo, P., Kiguba, R. and Mbaria, J. (2019). Ethnobotanical survey of medicinal plants used for renal and urinary disorders in East Africa. *Journal of Medicinal Plants Research*. **13**(7): 187–195.

Nwaehujor, C. O., Okoli, C. O. and Nweke, I. M. (2021). Phytochemical profile and biochemical evaluation of *Anthocleista grandiflora* leaf extract in rats. *African Journal of Biomedical Research*. **24**(3): 391–400.

Oboh, G., Olasehinde, T. A. and Ademosun, A. O. (2019). Antioxidant and nephroprotective potential of selected African medicinal plants. *Journal of Functional Foods*. 52: 293–304.

Okoli, C. O., Nwaehujor, C. O. and Adebayo, O. A. (2021). Comparative phytochemistry and renal activity of Anthocleista species used in West African herbal medicine. *Journal of Phytopharmacology*. 10(1): 33–45.

Olaleye, M. T., Akinmoladun, A. C. and Ogunboye, A. A. (2006). Nephroprotective effects of some medicinal plants in experimental models. *African Journal of Biomedical Research*. **9**(2): 119–124.

Olorunnisola, O. S., Adetutu, A. and Owoade, A. O. (2012). Sub-chronic administration of methanolic leaf extract of *Anthocleista vogelii* in rats: effects on haematological and biochemical parameters. *African Journal of Biotechnology*. **11**(57): 12049–12054.

Ozolua, R. I., Eboka, C. J. and Okhamafe, A. O. (2010). Evaluation of hematological and biochemical indices following administration of leaf extract of *Bryophyllum pinnatum* in rats. *Nigerian Journal of Physiological Sciences*. **25**(2): 107–113.

Ozolua, R. I., Idogun, S. E. and Okhamafe, A. O. (2009). Acute and sub-acute toxicological assessment of the aqueous extract of *Bryophyllum pinnatum* leaves in rats. *African Journal of Biotechnology*. **8**(15): 3737–3745.

Pooley, E. (2020). A Field Guide to Wild Flowers of KwaZulu-Natal and the Eastern Region. Durban: *Natal Flora Press*. 4: 21-89.

Rafieian-Kopaei, M., Nasri, H. and Bahmani, M. (2021). Herbal medicines for renal protection: Molecular mechanisms and safety considerations. *Journal of Renal Nutrition*. **31**(5): 450–463.

SANBI. (2023). *Anthocleista grandiflora* species profile. *South African National Biodiversity Institute Database*. 12: 12-35

Shah, R. and Patel, M. (2020). Anti-inflammatory pathways of flavonoids: Implications in kidney diseases. *Biomedicine and Pharmacotherapy*. 132: 110–118.

Thiv, M., Groger, A. and Kadereit, J. W. (2018). Phylogeny and biogeography of the genus Anthocleista (Gentianaceae) in Africa. *Botanical Journal of the Linnean Society*. **187**(2): 178–190.

World Health Organization (WHO). (2023). Global report on traditional and complementary medicine. Geneva: WHO Press.

Yakubu, M. T., Akanji, M. A. and Oladiji, A. T. (2003). Alterations in serum electrolytes in rats treated with aqueous extract of *Khaya senegalensis* stem bark. *Nigerian Journal of Biochemistry and Molecular Biology*. **18**(1): 35–40.