EFFECTS OF THE VACUUM LIQUID CHROMATOGRAPHY FRACTIONS OF THE LEAVES OF *Anthocleista djalonensis* A. CHEV (GENTINACEAE) ON BENIGN PROSTATIC HYPERPLASIA

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CERTIFICATION

This is to certify that this work was done by **Ogedengbe Taiwo Confidence** in the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City, Nigeria in partial fulfilment of the requirement of the award of the Doctor of Pharmacy Degree (Pharm. D).

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DEDICATION

This project is dedicated to the only One at whose feet I can lay everything God Almighty

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ABBREVIATIONS

VLC- Vacuum Liquid Chromatography

CC-Column Chromatography

BPH: Benign Prostatic Hyperplasia

IPSS- International Prostate Symptoms Score

PSA: Prostate Specific Antigen

MEL: 50:50, Ethyl actetate: Methanol and 100%Methanol Fraction

ELISA: Enzyme-Linked Immunosorbent Assay

DHT: Dihydrotestosterone

ROS: Reactive Oxygen Species

SASP: Senescence Associated Secretory Phenotype

DRE: Digital Rectal Examination

TRUS: Transrectal Ultrasound

SHBG: Sex-hormone Binding Globulin

ABSTRACT

The crude extract of *Anthocleista djalonensis* has been evaluated to have an effect on Benign Prostatic Hyperplasia. This study aims to evaluate the effect of chromatographic fractions of the plant on Benign Prostatic Hyperplasia. The aqueous extract of the leaves of the plant obtained, was subjected to Vacuum Liquid Chromatography, and the Ethyl acetate - Methanol Fraction and the 100% Methanol fraction (MEL) were bulked together and concentrated. Column Chromatography was performed on MEL; thereafter, thin-layer chromatography was performed, which showed that Fractions 2 and 3 contained similar constituents, hence were bulked together to afford Fraction A.

Nineteen male Wistar rats were divided into 5 groups and were used for this study. Group 1 was the normal control group, animals in this group were neither induced nor treated. Group 2 was the negative control group and were induced with BPH using 5 mg/kg testosterone acetate (dissolved in coconut oil) once daily via the subcutaneous route. Animals in group 3 and 4 were administered 25 mg/kg and 50 mg/kg of Fraction A respectively using the orogastric tube. Group 5 was the positive control group and were also administered 4 mg/kg finasteride (The reference) once daily using the orogastric tube after having dissolved it in a vehicle. This administration was done across 14 days. At the end of the 14th day, the rats were sacrificed, the prostate was harvested, and blood was collected. Histological evaluation of the prostate was done. Serum hormonal profile of testosterone and prostate-specific antigen was analysed using the Enzyme-Linked Immunosorbent Assay technique (ELISA). Also, prostate weight and volume was determined from the harvested prostate. Results showed that doses of fraction A impacted prostatic indices, and histological studies showed reduced hyperplasia. These advocates that the leave extract of *Anthocleista djalonensis* possess Anti-BPH activity supporting ethnobotanical studies. However, further studies

have to be carried out in order to ascertain its specific mechanism of action as well as toxicity studies in order to ascertain its safety.

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Benign prostatic hyperplasia (BPH), also known as nodular hyperplasia of the prostate is a non-malignant increase in the size of the prostate gland that frequently impacts older men and often leads to lower urinary tract symptoms (LUTS) such as reduced urine flow, frequent nighttime urination, and feeling of incomplete bladder emptying (Michael et al., 2021). The occurrence of BPH increases with age; both histological and clinical studies indicate that a significant number of men aged 60 to 70 or older exhibit some level of prostatic hyperplasia, with symptomatic cases contributing significantly to global morbidity.

As high as 80–90% histological incidence is still reported for men above 70 years in recent global reviews (Zi *et al.,* 2024). In 2021, approximately 112.5 million cases of BPH were estimated, reflecting a steady rise in burden since 1990 (Wei *et al.,* 2025). Among Nigerian men aged ≥44 years in a 2024 community-based study, a prevalence of 16.67% was recorded (Esomonu *et al.,* 2024), while a Ghanaian suburban population reported a LUTS-based prevalence of 42.3% among men aged 40–70 years (Cassell *et al.,* 2024).

1.2 Pathophysiology

The pathophysiology of benign prostatic hyperplasia (BPH) consists of both structural (static) and functional (dynamic) aspects. From a structural standpoint, there is a continual increase in the number of both stromal and epithelial cells, particularly in the transition (peri-urethral) zone of the prostate, which results in nodular enlargement. (Xu, G., *et al.,* 2024). This growth is stimulated by dihydrotestosterone (DHT), immune-mediated inflammation, and activation of local growth factors that enhance cellular proliferation and reduce apoptosis.

Chronic inflammation within the prostate gland results in the activation of fibroblasts and immune cells, causing fibrosis, remodeling of tissue, and the release of cytokines (for example IL-6, TGF-β, and TNF-α) that sustain the hyperplastic process. (Xu, G.,*et al.,* 2024). Additionally, oxidative stress plays a role by causing DNA damage and encouraging the senescence-associated secretory phenotype, which creates a microenvironment conducive to growth.

On the dynamic side, there is an increase in smooth muscle tone in the prostatic stroma and bladder neck resulting from the overstimulation of α-adrenergic receptors, leading to functional obstruction of urinary flow, even in the absence of significant gland enlargement. Disturbances in stromal–epithelial signalling pathways, the buildup of extracellular matrix, and changes in autonomic nerve supply all contribute to resistance at the bladder outlet. Ultimately, this progression results in compensatory hypertrophy of the detrusor muscle and lower urinary tract symptoms typically associated with BPH.

1.3 Causes

The underlying causes are multifaceted. The development of BPH is influenced by multiple factors, with androgen signalling playing a central role; specifically, dihydrotestosterone (DHT) produced by 5α-reductase encourages the growth of prostatic stromal and epithelial cells. (Michael *et al.,* 2024).

As individuals age, the balance between estrogen and androgen shifts, and the activation of estrogen receptor-α has been shown to promote stromal hyperplasia even further.

Long-term inflammation of the prostate, which involves the release of cytokines like IL-6 and TNF-α, causes repeated cycles of tissue damage and repair that contribute to nodular hyperplasia. The effects of oxidative stress and cellular aging intensify this process through the action of reactive oxygen species (ROS) and components of the senescence-associated secretory phenotype

(SASP). Disruption in the normal interactions between stromal and epithelial cells alters growth factor signalling, resulting in uncontrolled cell growth. Furthermore, conditions such as metabolic syndrome and hyperinsulinemia raise IGF-1 levels, which promote prostate growth through mitogenic pathways.

Lastly, heightened sympathetic activity and increased α-adrenergic signalling worsen smooth muscle hypertrophy and lead to functional obstruction.

These aspects are potential targets for both established and experimental treatments.

1.4 Risk Factors for Benign Prostatic Hyperplasia

B.P.H is influenced by both modifiable and non-modifiable risk factors.

- Advancing age: This is the most significant risk factor for BPH. At age 50, the prevalence and severity of BPH increases sharply. This is largely due to cellular senescence and hormonal changes that promote stromal and epithelial proliferation in the prostate.
- Genetic predisposition: Genetic predisposition plays a notable role, with studies showing that men with a family history of BPH have a risk of developing the condition, indicating possible genetic polymorphisms affecting androgen receptors and growth factor regulation (Michael *et al*., 2024)
- Metabolic syndrome, obesity, and insulin resistance: These conditions are strongly linked to BPH development. They promote sympathetic overactivity, chronic inflammation, and increased insulin-like growth factor-1 (IGF-1) signaling, which collectively stimulate prostatic growth (Michael *et al*.,2024)
- Lifestyle factors: Life style factors such as Physical inactivity as in sedentary lifestyle and smoking are associated with increased risk of BPH.

- Dietary factors: Dietary risk factors such as high fat and red meat (possibly due to polycyclic aromatic hydro- carbon formed from cooking), alcohol and caffeine, high dose of vitamin C, Niacin and Zinc supplements, may be linked to increased risk of developing BPH.

1.5 Signs and Symptoms of Benign Prostatic Hyperplasia

The signs and symptoms of BPH are broadly classified into obstructive and storage categories which show bladder outlet obstruction and compensatory change to the bladder. (Roehrbon, 2022). These factors were also outlined by Rowden (2023).

1. Obstructive Symptoms;

These occur due to mechanical compression of the urethra by the enlarged prostate and increased smooth muscle tone in the bladder neck and prostatic urethra. Common manifestations include:

a. Hesitancy: Delay in initiating urination.

b. Weak or intermittent urinary stream: Reduced force of urine flow.

c. Straining during micturition: Need to apply effort to void.

d. Prolonged urination time.

e. Incomplete bladder emptying: A persistent sensation of residual urine after voiding.

f. Post-void dribbling: Leakage of small amounts of urine after urination.

2. Storage (Irritative) Symptoms;

These result from detrusor overactivity as a result of prolonged bladder outlet obstruction and include:

a. Increased urinary frequency.

b. Nocturia: Frequent need to urinate at night.

c. Urgency: Sudden compelling desire to void.

d. Urge incontinence: Leakage of urine associated with urgency.

If left untreated, BPH may progress to acute urinary retention, recurrent urinary tract infections, hematuria, bladder stones, and hydronephrosis due to chronic obstruction.

1.6 Diagnosis

The diagnosis of BPH involves a combination of clinical evaluation, physical examination, diagnostic investigation and clinical assessment, this is to rule out other possible causes of lower urinary tract symptoms and confirm prostate enlargement.

Past medical History and assessment of symptoms: This involves taking a detailed medical history on urinary symptoms such as nocturia, hesitancy, urgency, frequency and incomplete emptying. The International Prostate Symptom Score (IPSS) is widely used to assess disease impact on quality of life.

Physical Examination: This involves the Digital Rectal Examination (DRE) technique, a key initial step that allows the examination of prostate size, symmetry, consistency, tenderness and exclusion of nodules suggestive of malignancy. In this technique, the prostate is felt for any irregular or hard areas and examined if it is larger than expected for a particular age by inserting a gloved, lubricated finger through the rectum. This method, though subjective, gives information to guide further evaluation.

Laboratory investigations: These include Prostate Specific Antigen (PSA) test, urinalysis and renal function test. Serum PSA serves as a biomarker for various prostate conditions including BPH, prostatitis and prostate cancer. Elevated PSA level is usually seen in these prostrate conditions and that is due to an enlarged prostrate gland.

Ultra sound technique: Ultra sound techniques like transrectal ultrasound (TRUS) and pelvic ultrasound help in measuring prostate volume. TRUS is an invasive procedure that employs high frequency waves to scan the prostate in order to check for the size.

Urodynamic and additional tests: Uroflowmetry and post void residual could be used when the diagnosis remains uncertain. While the former addresses quantification of urinary flow rate, the latter addresses the efficiency of the bladder to empty.

Symptom scoring tools for measuring BPH: Asides physical and laboratory information, score tools are used to assess the severity and impact of LUTS on quality of life as well as response to medications. The most widely used is the International Prostate Symptom Score (IPSS) (Deters, 2023), derived from American Urological Association (AUA). IPSS consist of seven questions addressing both storage and voiding symptoms – such as 1. weak urinary stream, 2. straining, 3. intermittency, 4. frequency, 5. nocturia, 6. urgency, 7. incomplete emptying, and an additional quality of life question that assesses how bothersome the symptoms are to the patient. Each question is rated on a scale from 0 to35, with mild {0 to 7}, moderate {8 to 19}, or severe {20 to 35} symptom severity (Deters, 2023). The AUA symptom index, on which the IPSS is based, includes the same questions but omits the QOL assessment, while the IPPS is internationally validated and more widely adopted.

1.7 Management of Benign Prostatic Hyperplasia

The management of BPH depends on the severity of symptoms, presence of complications, prostate size, patient preference. The goals of treatment focus on decreasing prostate size and relaxing smooth muscle (Lepor, 2005), and alleviating symptoms, improving quality of life, and preventing complications. Treatment options vary from watchful waiting (non-interventional) to pharmacological and surgical therapy.

Watchful waiting (Non-interventional): Men with mild symptoms (IPSS< 7) or minimal bother are often managed with this option which involves regular monitoring of the patient and lifestyle modifications. These include decreasing caffeine and fluid consumption in evenings, decreasing caffeine consumptions, practicing time voiding, avoiding medications that may worsen urinary symptoms such as decongestant and anticholinergics (Ng.*et al* 2024).

Pharmacological Therapy: This is usually the mainstay for men with moderate to severe symptoms. These include:

Alpha-1 receptor blockers: Alpha 1 receptors are located in the smooth muscle of the prostrate, bladder and proximal urethra. Alpha 1 blockers like Alfuzocin, prazocin, tamsulocin, derazocin, silodosin, work by inhibiting these receptors which leads to relaxation of the smooth musclesin the lower urinary tract. This reduction in muscle tone helps decrease bladder outlet resistance and enhances urinary flow in men with BPH (Bortnick *et al* 2023). Common side effects include orthostatic hypertension, dizziness, nasal congestion, dizziness, fatigue and occasional ejaculatory dysfunction. Selective alpha 1A blockers like tamsulocin and silodosin are preferred for BPH because it targets the prostate more specifically and minimises the side effects of hypotension. Alpha 1 blockers are first line therapy for men with moderate to severe BPH symptoms who do not have markedly enlarged prostates. They are often used in combination with 5 alpha reductase inhibitors in men with larger prostates as they do not shrink the prostate or halt long term disease progression but just provide rapid relief of symptoms. (McVary *et al*.,2021; Gratzke *et al.,* 2023).

5-Alpha reductase inhibitors: The enzyme 5 Alpha reductase is responsible for the conversion of testosterone to dihydrotestosterone. Drugs like finasteride, dutasteride and botulinum toxin, inhibit this enzyme leading to a substantial decline in DHT levels hence reduction in prostate volume and

reduced risk of urinary retention. These agents are most effective in men with significantly enlarged prostate, lowers the likelihood of urinary retention (Gratze *et al.,*2023)

Phosphodiesterase 5 inhibitors: such as Tadalafil improves LUTHS while simultaneously improving erectile dysfunction. Also, the use of antimuscarinic agonists for patients with overactive bladder coexisting with BPH has also demonstrated high efficacy. (Gratzke *et al.,* 2023).

Surgical Therapy: Surgical treatment is recommended when men develop complications such as recurrent urinary retention, bladder stones, renal impairment, hematuria or when symptom persist despite medical therapy. The Transurethral Resection of the Prostate (TURP) is the gold standard surgical procedure for BPH treatment. During TURP, a resectoscope is inserted through the urethra to remove the obstructive prostatic tissue surrounding the urethra channel. The tissue is cut or vapourised using a heated wire loop or laser energy (Gratze *et al.,* 2023).

Phytotherapy

Phytotherapy is the use of plant-derived extracts or herbal preparations in the management of certain conditions, in this case BPH. It remains popular in the world mostly in Africa owing to its accessibility, affordability and availability, Also, the fact that many medications may produce certain undesirable effects especially in the elderly (Bortnick *et al.,* 2020) necessitates the need for the exploration of more drugs. Some of the phytotherapies explored for the management of BPH include:

I. Cucurbita pepo

Cucurbita pepo commonly called field pumpkin is of the Order; *Cucurbitales*, Family; *Cucurbitaceae*, Genus; *Cucurbita*, and Species; *Cucurbita pepo.*

Its seed is rich in zinc and phytosterols reported in managing lower urinary tract symptoms and to improve urinary flow through its anti-inflammatory and anti-androgenic properties.

II. Hypoxis hemerocallidea

Hypoxis hemerocallidea commonly called African Potato or Star Lily is of the Order; *Asparagales*, Family; *Hypoxidaceae*, Genus; *Hypoxis*, and Species; *Hypoxis hemerocallidea*

It is also rich in Phytosterols specifically B-sitosterol which inhibits prostatic cell proliferation and dihydrotestosterone activity which inhibits urinary symptoms. (Adewoyin *et al*., 2022).

III. Prunus africana

Prunus africana commonly known as African Prune tree is of Order; *Rosales*, Family; *Rosaceae*, Genus *Orunum* contains Phytosterols specifically B-sitosterol which have demonstrated anti-proliferative, anti-inflammatory and anti edematous actions. These mechanism help improve urinary outflow and reduces post void residual urine in men with BPH (Ndung'u *et al.,* 2024).

IV. Serenoa repens

Serenoa repens commonly known as Saw Palmetto of Order: *Arecales*; Family: *Arecaceae*; Genus: *Serenoa Hook*; Species: *Serenoa repens.*

It is one of the most widely studied plants as a phytotherapy for B.P.H. Its lipidosterolic extract acts by inhibiting 5 alpha-reductase, exerting anti-inflammatory effects, and blocks dihydrotestosterone binding to androgen receptors within prostatic tissue. Some studies show higher efficacy compared to alpha blockers (Nguyen *et. al.,* 2023).

V. Urtica dioca

Urtica dioca root commonly called stinging nettle is thought to possess multiple therapeutic activity, inhibits prostate cell proliferation and reduces prostatic inflammation by interfering with sex-hormone binding globulin (SHBG).

Phytotherapeutic agents are well tolerated with lesser side effects and adverse effects compared to conventional medicines, however, Standardisation is a key limitation.

1.9 Anthocleista djalonensis

Anthocleista djalonensis (A. Chev) is a medicinal tree native to West Africa. It is commonly called cabbage tree and referred to as “Ewe Shapo" by the Yorubas of south western Nigeria; as oduku by the ibo; and as Oyinmwin’ wi-uwu by the Binis (Enoghase *et al.,* 2025).

1.9.1 Taxonomy

Taxonomic Rank and Classification

Kingdom: Plantae

Subkingdom: Tracheobionta (Vascular plants)

Superdivision: Spermatophyta (Seed plants)

Division: Magnoliophyta (Angiosperms)

Class: Magnoliopsida (Dicotyledons)

Subclass: Asteridae

Order: Gentianales

Family: Gentianaceae (formerly, loganiaceae)

Genus: *Anthocleista* R. Br.

Species: *Anthocleista djalonensis A. Chev*.

1.9.2 Description and Distribution

Anthocleista djalonensis A. Chev, Family; Gentianaceae, is a deciduous tree, about medium to large and grows to about 15 to 20 metres, with bows about 40 centimetres in diameter and twigs with two erect spikes and tiny cushions above the leaf axis (Jensen et al., 2001) with a light grey to brown bark. Its leaves are large and opposite, elliptic to ovate and are about 45cm in length,

glossy green and have midribs and short petiole. The flowers range from white to pale yellow, emit a pleasant fragrance, and are clustered in terminal panicles. They are tubular and possess four to five lobes. The fruit is a round berry, which is green when immature and becomes yellowish-brown as it ripens, and contains many small seeds encased in a soft pulp. The wood is soft and lightweight.

1.9.3 Geographical Distribution

Anthocleista djalonensis originates from the tropical regions of West and Central Africa. It can be found in several countries, including Nigeria, Ghana, Sierra Leone, Cameroon, Côte d'Ivoire, the democratic Republic of Congo and Liberia.

This species flourishes in humid tropical environments, particularly within lowland rainforests, along riverbanks, at forest peripheries, and in areas of secondary vegetation. It favors well-drained, nutrient-rich soils and is commonly located in regions characterized by high rainfall and moderate levels of sunlight. Although, it can also be found in normal terrestrial environments.

In Nigeria, it is particularly prevalent in the rainforest and derived savanna regions, where it grows in wild settings as well as traditional home gardens.

Figure 1: Picture of *Anthocleista djalonensis* leaves.

1.10 Ethnomedicinal Uses

Anthocleista djalonensis A. Chev. Is an important specie within the *Anthocleista* genus and it's widely recognised in African traditional medicine for its broad therapeutic uses. Extracts of its leaves, roots, and stem bark are traditionally applied in managing a variety of conditions, including wounds, constipation, diarrhoea, dysentery, abdominal discomfort, hepatitis, jaundice, liver disorders such as cirrhosis, fungal skin infection, filarial infestations, acute inflammation, and skin boils. (Enoghase *et al.,* 2025).

1.11 Medicinal Properties/ Pharmacological Activities

Anthocleista djalonensis A.Chev.(Gentianaceae) is widely recognised in African traditional medicine because of its broad pharmacological activities. Some of these properties include:

A. Antidiabetic Activity: *A. djalonensis* is commonly administered as a decoction or as an acqueous/alcoholic macerate, taken orally for the management of diabetes in several west and Central African countries, including Guinea, Nigeria, Togo, Ghana and Cameroon (Adebayo *et al.,* 2022). Scientific evidence aligns with its traditional use, demonstrating significant hypoglycemic effects across different plant parts- particularly the leaves, stem bark and roots- in both invitro and in-vivo experimental models (Adebayo *et al.,* 2022). Extracts of this plant have shown notable alpha amylase activity, with the acqueous methanol extract exhibiting inhibition at 1 Ml of 250 mg/Ml concentration. Comparative evaluations among *anthocleista* species indicate that the plant displays superior antidiabetic activity suggesting concentration or potency of bioactive constituent responsible for glucose lowering action.(Olubuhemin *et al* 2013; Okonkon *et al*, 2012)

B. Antiplasmodial activity: Both traditional and scientific evidence has shown that *A. Djalonensis* can inhibit plasmodium falciparum and plasmodium berghi. A dose dependent

antiplasmodial activity at dose range of 50 to 300 mg/kg has been shown in *invitro* studies (Enoghase *et al.,* 2025). Methanol and aqueous extract of the leaves and barks have shown activity against plasmodium falciparum (Kouadio *et al*., 2023, Agyare C. *et al*., 2024).

C. Antioxidant and Organ-Protective Activity: Recent studies have demonstrated that aqueous methanolic extract of the leaf and bark of *A. djalonensis* has strong antioxidant activity both *in vitro* and *in vivo* (It demonstrated free-radical scavenging ability, reduction of lipid peroxidation, and protection against chemically induced hepatic and cardiac damage in animal models (Taiwe *et al.,* 2021.) Its antioxidant property could be owing to its phenolic and flavonoid constituents.

D. Anti-inflammatory and Analgesic Activity: Anti-inflammatory compounds account for nearly half of all analgesic agents, as they relief pain primarily by suppressing inflammation and associated tissue swelling. In Nigeria, a traditional cold infusion prepared from stem bark combined with eight other herbs is commonly taken- two table spoon daily- for the management of asthma (Enoghase *et al.,* 2025).

   Additionally, topical administration of a poultice made from the plant is used in treating wounds and various rheumatic pain in ethnomedicine. The methanolic root extract of the plant exhibits both anti-inflammatory and analgesic activities, acting on central and peripheral pathways to reduce neurogenic and inflammatory pains. (Enoghase *et al.,* 2025).

E. Anthelmintic Activity: The leaf, bark or roots of *Anthocleista djalonensis* is cooked in water and drunk, and traditionally used in Nigeria as a purgative. Several ethnobotanical and pharmacological studies have reported that *Anthocleista djalonensis* exhibits significant anthelmintic activities, supporting its traditional use for treating intestinal worm

infestations and constipation in West Africa. These effects are primarily attributed to the presence of iridoid glycosides, alkaloids, saponins, and tannins, which interfere with the neuromuscular activity of helminths, interfering with calcium ion channels and promote intestinal motility, leading to flaccid paralysis (Adeyemi *et al*., 2020, Adebayo *et al.,* 2022). Adeyemi *et al.,* demonstrated this using ethanolic and aqueous extract of the plant on Pheretima post-human where a dose dependent paralysis and death of the worm is seen.

F. Purgative and Laxative activity: Decoctions of *A. djalonensis* bark and root are used as purgatives or mild laxatives to relieve constipation and gastrointestinal discomfort traditionally. Adebayo *et al.,* 2022 demonstrated this. *A. djalonensis* does this by stimulating muscle contraction via cholinergic and serotonergic receptors.

G. Reproductive activity: In south west Nigeria, the plant has a traditional reputation for enhancing male reproductive health- including boosting libido, promoting penile erection, raising sperm count and thereby improving overall male fertility (Olowokudejo *et al.,* 2008, Adebayo et al., 2022). Experimental studies using wistar rats shows that it enhances spermatogenesis and improves reproductive hormone profile. It does this through modulation of the hypothalamic pituitary axis. (Enoghase *et al.,* 2025) It enhances aphrodisiac activity (Kouadio *et. al,* 2023). It is proposed that the underlying mechanism is through enhancement of nitric oxide signalling and secretion of testosterone.

1.12 Phytochemistry

Anthocleista djalonensis A.Chev (Gentianaceae) has many phytochemical constituents which are responsible for its diverse pharmacological activities. The iridoids and xanthones are considered the major bioactive classes. Iridoids and seco iridoids glycosides like

sweroside, sweroside aglycone and loganin have been identified in the stem barks and leafs. (Adebayo *et., al* 2022, Kouadio *et al.,* 2023).

Xanthones derivatives including mangiferin, anthocleistone and 1,8-dihydroxy-3-methylxanthone have been isolated from the bark and root extracts. (Taiwe *et al.,* 2021). It also contains phenolic compounds such as rutin, quercetin and kaempferol. Its roots and bark extract also contains saponins and alkaloids including anthocleistine and gentianine (Adebayo *et al.,* 2022).

The plant also contains other secondary metabolites like Triterpenoids such as ursolic acid, betulinic acid and lupeol. It also contains phytosterols such as B. sitosterol and stigmasterol which is thought to be responsible for its anti-BPH activity. GC - MS analyses have revealed fatty acids (like palmitic, linoleic acid), simple sugars and trace minerals.

1.13 Justification for the Study

Benign Prostatic Hyperplasia remains one of the most common urologic conditions affecting older men, substantially impairing quality o life through lower urinart tract symptoms (LUTS) and related complications. Although treatments such as alpha adrenergic blockers and 5 alpha reductase inhibitors can relieve LUTS, their long- term use is frequently associated with unwanted side effects- including dizziness, orthostatic hypotension, sexual dysfunction, and poor adherence by patientssssss (Gravas *et al.,* 2023, Mcvary KT *et al.,* 2021). For this reason, safer, more effective, and affordable alternative therapies derived from medicinal plants with scientifically validated efficacy and safety profiles are needed.

Anthocleista djalonensis A. Chev (Gentianaceae) is widely used in African traditional medicine for the treatment of metabolic, inflammatory, and reproductive disorders.

Preliminary pharmacological investigations have demonstrated that extracts of A. djalonensis possess anti-inflammatory, antioxidant, antidiabetic, hepatoprotective, and androgen-modulating properties (Kouadio *et al.,* 2023, Taiwe *et al.,* 2023, Ogunboye *et al.,* 2022). Since oxidative stress and chronic inflammation are central mechanisms in the pathogenesis of BPH, these already established pharmacological activities suggest that *A. djalonensis* may provide therapeutic benefit in managing Benign Prostatic Hyperplasia (BPH).

Furthermore, the plant is rich in iridoids, xanthones, and triterpenoids, compounds known to inhibit 5-α-reductase and modulate hormonal balance which are the key pathways implicated in prostate enlargement (Taiwe *et al.,* 2021, Ogunboye *et al.,* 2022). However, despite extensive ethnomedicinal use, there remains limited scientific validation of its efficacy in BPH management and the specific bioactive fractions responsible for its effects. Therefore, this study is justified as it seeks to scientifically evaluate the effect of chromatographic fractions of aqueous extract of *Anthocleista djalonensis* on experimentally induced BPH. The findings will give valuable insight into the pharmacological potential *A. djalonensis*, contribute to the development of safer plant-based therapeutics, and substantiate its ethnomedicinal claims with empirical evidence.

1.14 Objectives of the study

This study aims to evaluate the effect of chromatographic fractions of aqueous extract of *Anthocleista djalonensis* on Benign Prostatic Hyperplasia.

Specific objectives:

1. To prepare and separate the aqueous fraction of the leaf extract of *Anthocleista djalonensis* using chromatographic techniques (Vacuum Layer Chromatography, Column Chromatography and Thin Layer Chromatography).
2. To evaluate the effects of the fraction A gotten on prostate size, prostate volume and histomorphology in experimentally induced BPH.
3. To assess the influence of the extract and the fraction on serum testosterone and Prostate Specific Antigen (PSA) levels in treated animals.
4. To compare the efficacy of the fraction A gotten to standard Anti-BPH drug (Finasteride).

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 Equipment and Apparatus

Soxhlet apparatus, electric heating mantle, jumbo condenser, water bath, Oven, micro pipettes, test tube and test tube racks, refrigerator, round bottom flask, beakers, Conical flasks, freezer, porcelain dishes, stirrer, glass jars, measuring cylinders, Thin layer chromatography plates, Column, ruler, pencils, retort stand, mortar and pestle, Spray can, spatula, cafes, Chamber, scrapper, digital weighing balance, plain bottles, sample bottles, Chamber, Vacuum pump, Buchner Funnel, Rotary evaporator, Forceps, Glass Jar, Centrifuge,

2.1.2 Reagents and Chemicals

Dichloromethane, Ethyl acetate, Methanol, Silica gel, Distilled water, Concentrated Sulphuric acid, Formalin, n-hexane, and Chloroform.

2.1.3 Consumables

Cotton wool, filter paper, Chloroform, Face masks, gloves, growers pellets, syringes, cage beddings, Detergent, Hand sanitisers, and Dettol.

2.2 Methodology

The extract was obtained using soxhlet apparatus. The aqueous extract was obtained by partitioning the extract with Dichloromethane and water. The aqueous extract was concentrated, weighed and subjected to vacuum liquid layer chromatography.

2.2.1 Chromatography and Fractionation

- Vacuum Liquid Chromatography (VLC):

  VLC was performed as described by Amechina *et al*., (2022). The concentrated aqueous extract of the leaves of *Anthocleista djalonensis* (12g) was adsorbed onto silica gel and triturated to form a dry, free-flowing powder. The Buchner flask in the VLC column was wet-packed with silica as a slurry in the starting solvent (Dichloromethane) under light vacuum to obtain a uniform bed. Elution was performed under suction with 100mL of 100% Dichloromethane, Dichloromethane – Ethyl acetate (1:1), 100mL of 100% Ethyl acetate, Ethyl acetate – Methanol (1:1), and 100mL of 100% methanol in succession.

  Out of the 5 fractions from the VLC, fractions 4 and 5, the Ethyl acetate – Methanol (1:1) and 100 mL of 100% methanol (MEL fraction) were combined and concentrated using the rotary evaporator. The concentrated MEL fraction was weighed (8.91g).

- Column Chromatography (CC):

  Column chromatography was performed on the MEL fraction as described by Ofeimum *et al*. (2014) and Akinlabi *et al*. (2020). MEL fraction (8.91g) was chromatographed on a silica gel (which served as the stationary phase) column after a slurry was formed with n-hexane and silica gel. Elution was performed in order of increasing polarity with Dichloromethane (DCM) and Methanol using 300mL in each ratio of 80:20 (Test tubes 1 to 9), 70:30 (test tubes 10 to 22), 60:40 (test tubes 23 to 31), 50:50 (Test tubes 32 to 41),

40:60 (Test tubes 42 to 50), 30:70 (Test tubes 51-63), 20:80 (Test tubes 64-74), up to 10:90 (Test tubes 75-87) and 100% methanol (Test tubes 87-99).

- Thin Layer Chromatography (TLC):

  Thin Layer Chromatography analyses of the various column fractions were carried out using a TLC plate with DCM and Methanol in a ratio 9:1, and 7:3 for Test tubes 1-9, 10-22 and 23 to 31; 1:1 for Test tubes 32 to 41; and 7:3 for Test tubes 42 to 99 as developing solvent system in a TLC chamber. The fractions were spotted in precoated TLC plates and allowed to air-dry; thereafter, they were placed in their respective solvent systems. The TLC plates were sprayed with 100% Concentrated Sulphuric acid and heated for 5 minutes at 110°c in an oven. The spots were noted, and the retention factor values were determined. Fractions with similar retention factor values were pooled together as A (Fractions 2 and 3 in test tubes 1-22), B (23 to 41), C (54 to 57), and D (75 to 78). These fractions were then concentrated and weighed. Fractions 2 and 3 (A) weighed 5.06g and were used for this experiment/study.

2.2.2 *In vivo* Studies

2.2.2.1 Experimental animals

Nineteen (19) male Wistar rats weighing between 119 kg to 217 kg were acquired from the animal house at the Department of Pharmacology, University of Benin, and acclimatised for two weeks under standard laboratory conditions (temperature 21 to 25°C, access to standard growers pellets diets and clean water, ventilated plastic cages and wood shavings as beddings). Before any Experimental procedure was commenced, ethical approval was obtained from the Ethical Review Committee of the Faculty of Pharmacy.

2.2.2.2 Experimental designs and groupings

This was done according to the method described by Ofeimum *et al*. (2021). Nineteen male Wistar rats were divided into 5 groups. On day 15, groups 2 through 5 were induced with BPH using 5 mg/kg testosterone acetate (dissolved in coconut oil) once daily via the subcutaneous route. Animals in group 5, the positive control group were also administered 4 mg/kg finasteride (The reference) once daily using the orogastric tube after having dissolved it in a vehicle. Animals in groups 3 and 4 were administered 25 mg/kg and 50 mg/kg of Fraction A, respectively, using the orogastric tube. Animals in group 1, which was the normal control group, were neither induced nor treated. Animals in group 2, the negative control group were administered only 5 mg/kg testosterone acetate dissolved in coconut oil subcutaneously. This administration was done across 14 days. The body weights were taken at baseline and at the conclusion of the 14 days. Animals were observed daily for food intake, behaviour and clinical signs, and mortality was recorded.

Table 1: Groupings of animals in the experiment to test for the anti-BPH activity of the MT fraction of the aqueous leaf extract of *Anthocleista djalonensis* A.Chev.

Groups	Number of Animals	Treatment Administered
1	3	Normal (No treatment was given)
2	4	Testosterone 5mg/kg subcutaneously.
3	4	Testosterone 5mg/kg subcutaneously + 25 mg/kg MT fraction orally for 14 days.
4	4	Testosterone 5mg/kg subcutaneously + 50 mg/kg MT fraction orally for 14 days
5	4	Testosterone 5mg/kg subcutaneously + 4mg/kg Finasteride orally for 14 days.

2.2.3 Sample collection and Analysis.

Twenty-four hours after the last administration, the rats underwent an overnight fast. After which they were weighed, anaesthetised (in a chamber containing chloroform), and dissected. Blood was collected into plain bottles from the inferior vena cava and allowed to stand for 45 minutes at room temperature, after which centrifuged to separate the serum. The serum was then used for hormonal assays (Ttestosterone and Prostate Specific Antigen).

The prostate glands of the rats were also harvested and weighed. After which, the prostate volume was determined. The results from the body weight of the rats and the weight of the prostate glands were used to get the prostate index which gives an understanding of the physiological effects of the administered drugs. The prostate glands were then fixed in 10% neutral buffered formalin for histology.

2.2.4 Determination of Prostate indices.

The final body weight of individual rats were determined before they were sacrificed. After which dissected and the prostate gland was carefully removed and placed in a clean beaker on a weighing scale. The average weight of the prostate gland for each group was accurately determined from the weights of the prostate glands and recorded correctly. After this, the prostate gland was moved into a 10mL measuring cylinder containing 5mL of water measured using a 5mL syringe. The displacement of water was measured in order to determine the prostate volume.

The prostate index (P.I.) was calculated by dividing the final body weight of the animals by the weight of the prostate, and the mean P.I for each group was calculated.

2.2.5 **Determination of Serum Hormonal Profile.**

The levels of Prostate Specific Antigen (PSA) and testosterone were determined using the serum collected. This was done using the commercial Enzyme-Linked Immunosorbent Assay (ELISA) Test kits (Ofeimun *et al*., 2021).

2.2.6 Histological Studies.

For the histological studies, harvested prostate glands of the rats were preserved in 10% neutral buffered formalin. The tissue was dehydrated with upgraded alcohol concentration, cleared using xylene and embedded in paraffin. It was thereafter sectioned to about 5 mm thickness, rewaxed with xylene and rehydrated in ascending alcohol concentration and stained using hematoxylin in eosin. Xylene was used to clar the section after rehydration and mounted with dibutylphthalate polystyrene using cover slips for microscopic evaluation (at ×40 and × 100) and the results were photographed for proper documentation and interpretation. (Imade *et al*., 2024, Ofeimun *et al*., 2023).

2.2.7 **Statistical Analysis**

Experimental results were stated as mean ± SEM (Standard Error of Mean), n=3. Data analysis and presentation were done using GraphPad Prism (version). Statistical evaluation between groups was done using one-way Analysis of Variance (ANOVA) and subsequently Tukey--kramer

multiple comparison test to compare means in cases of statistically significant difference. At p-values less than 0.05, results were considered to be statistically significant.

CHAPTER 3

3.0 RESULTS

3.1 Thin Layer Chromatography Profile of Fractions Following Column Chromatography

TLC analysis was carried out on Fractions 2 and 3 obtained from Column Chromatography using Dichloromethane and Methanol as solvent systems in the ratio 9:1 and 1:1, respectively.

As shown in both plates 3.1 and 3.2, Fractions 2 and 3 exhibited similar spot patterns and RF (Retention factor) values, indicating that they contained components of comparable polarity. Hence, Fractions 2 and 3 were pooled together to afford Fraction A for further analysis.

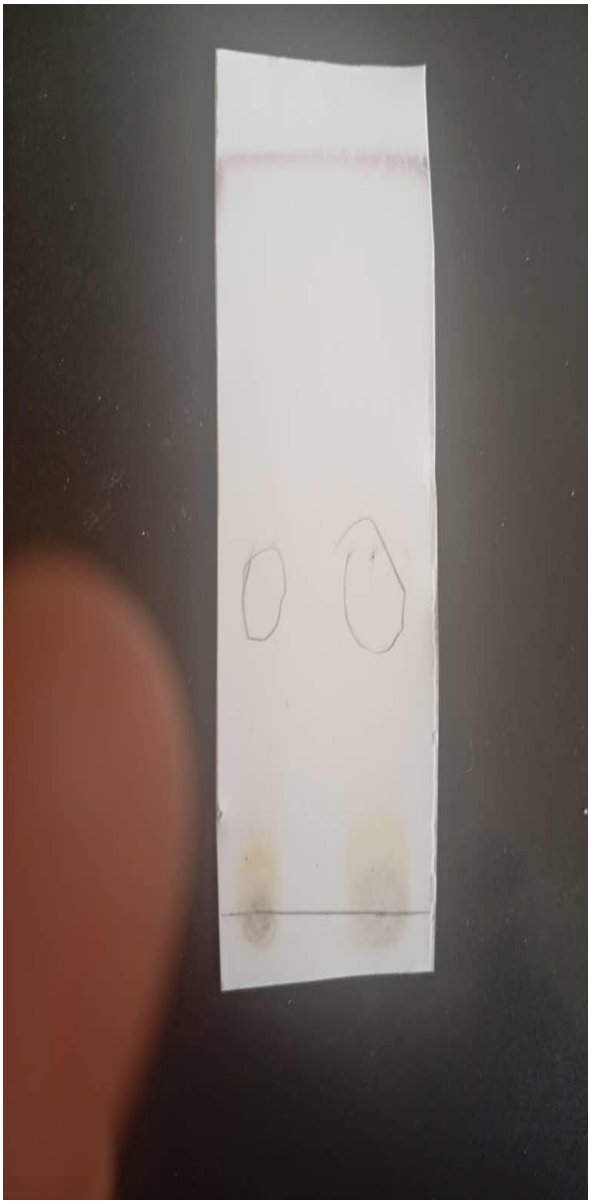

Plate 3.1 Plate 3.2

Plates 3.1 and Plate 3.2 (TLC plates) showing the chromatographic profiles of fractions 2 and 3 obtained from Column Chromatography.

3.2 Effects of Fraction A of the Aqueous extract of the leaves of *Anthocleista djalonensis* on Prostatic Parameters

Animals in group 2-the negative control group had the highest mean P.I value, while those in group 5, the normal control group showed the lowest P.I value as shown in table 2. However, those in group 2 and 3, that were giving 25 mg/kg and 50mg/kg of the extract showed similar Prostate Index values as seen in the reference group- group 5. The results from the prostate weight, prostate volume and prostate index revealed a significant difference ($p < 0.05$) among the treatment groups using one-way ANOVA.

For the prostate weight, one way ANOVA showed $p<0.05$, results from Tukey-kramer showed a statistically significant difference ($p<0.05$) in the negative control group vs the group administered 25mg/kg of Fraction A, $p< 0.001$ compared to the group administered 50 mg/kg Fraction A , and <0.0001 compared to the normal control group.

For the prostate volume, one-way ANOVA analysis showed a significant difference ($p<0.001$) and also for Turkey-karma analysis in the negative control group versus the other groups as seen in figure 3.

For the prostate index, one-way ANOVA showed $p<0.001$. Further analysis using Tukey-kramer showed a statistically significant difference $p<0.001$ between the negative control group versus the positive control, normal control and 25 mg/kg of Fraction A Group as shown in figure 4.

Table 2: Effects of Fraction A of the Aqueous Extract of *Anthocleista djalonensis* on Prostatic Parameters

Groups	Mean prostate weight (kg)	Mean body weight (kg)	Mean prostate index (%)	Mean prostate volume (mL)
1	0.1102±0.01	136.3±17.1	0.0808±0.01	0.09±0.02
2	0.3437±0.03	148.7 ± 1.62	0.2359±0.02	0.53±0.12
3	0.1978±0.02	121±4.0	0.1635±0.02	0.15±0.10
4	0.1525±0.02	150.5±18.8	0.1013±0.01	0.20±0.00
5	0.1279±0.01	134.3±8.2	0.0951±0.01	0.17±0.04

Group 1: Normal Control group; Group 2: Negative control; Group 3: 25 mg/kg of Fraction A; Group 4: 50 mg/kg of Fraction A; Group 5: Positive control.

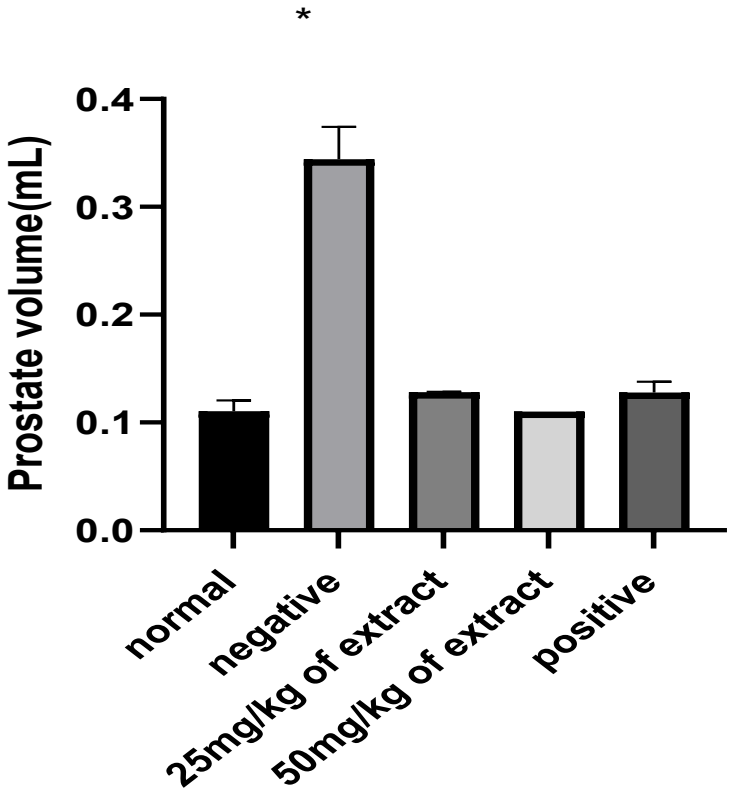

Figure 2: **Effect of Fraction A on Prostate volume (mL) (P*Vs Positive, Normal and 50mg/kg), and**

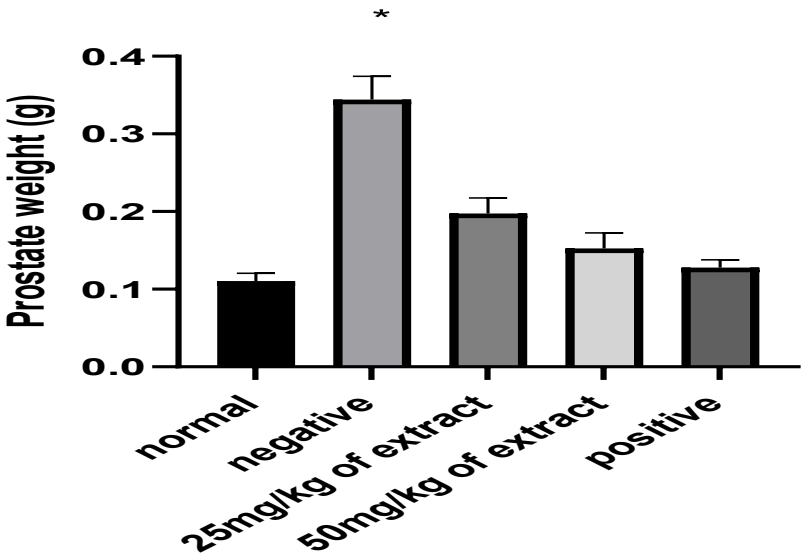

Figure 3: **Effect of Fraction A on Prostate weight(g) (P*Vs Positive, Normal and 50mg/kg)**

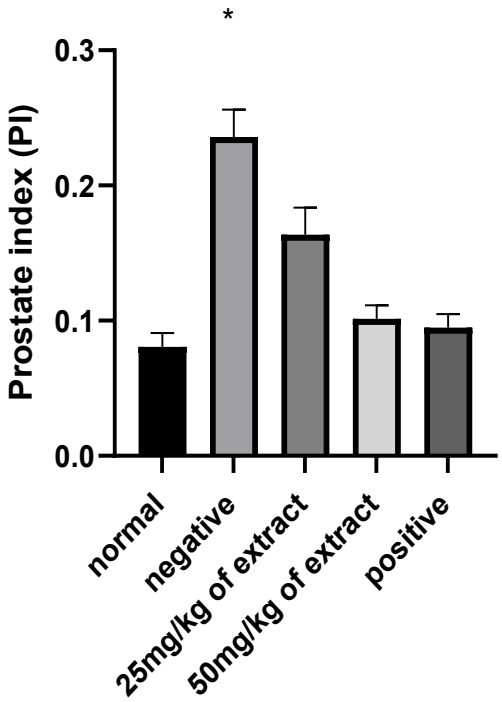

Figure 4: Effect of fraction A on Prostate index
(P* VS positive , normal , 25mg/kg of extract and 50mg/kg of extract)

3.3. Histological Findings

It was observed from the results that the prostate of the animals in the normal control group (Figure 5) showed normal acini, with wide lumina and no visible lesions. The prostate of the animals in the negative control group (Figure 6) showed acini with exaggerated glandular and crowding folds, degeneration of epithelial cells, and stroma thickening. The prostate tissue of rats administered 25 mg/kg of fraction A (Figure 7) showed reduced irregular and moderately distended acini with lesser degeneration and glandular folds. Those administered 50 mg/kg of the extract (Figure 8) showed larger acini with glandular foldings and minimal degeneration. The positive control group (Figure 9) showed nearly normal and well-defined epithelial folds with no obvious degeneration and no visible lesions.

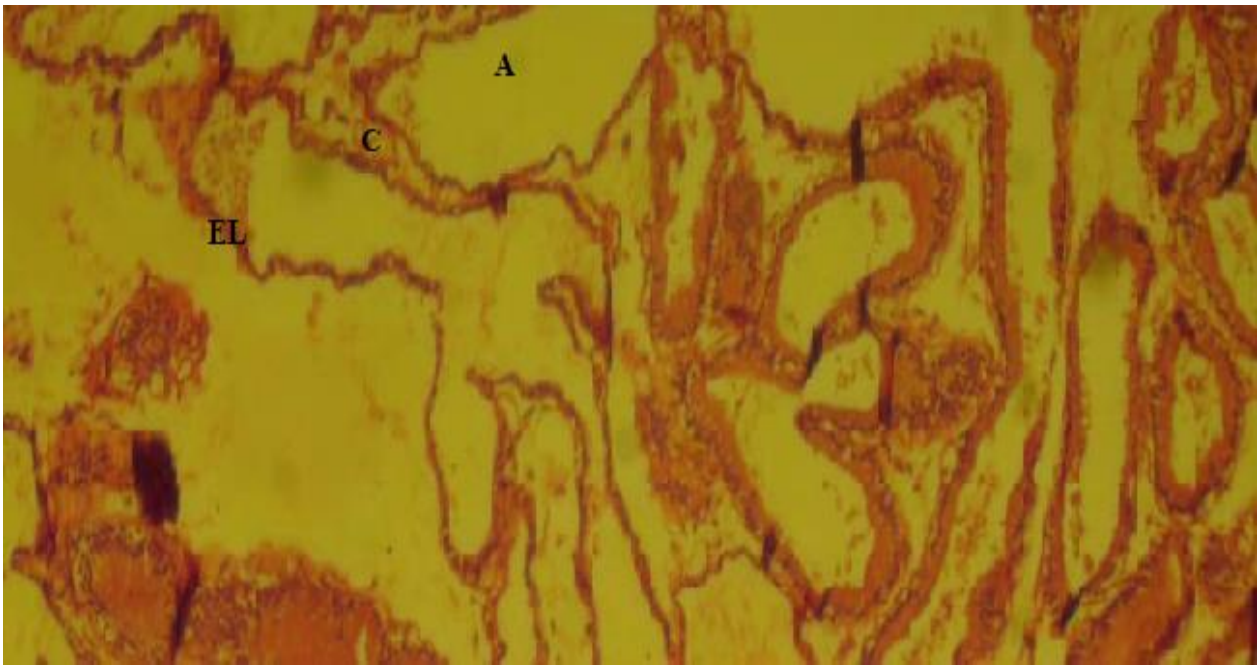

Figure 5: Micrograph of the prostate tissue in the Normal control group. Acini(A) are distinct, well-formed and normal. The lumen is wide and clear and the thickness of the epithelial lining (EL) appeared normal. The connective tissue (C) is also normal. (x40)

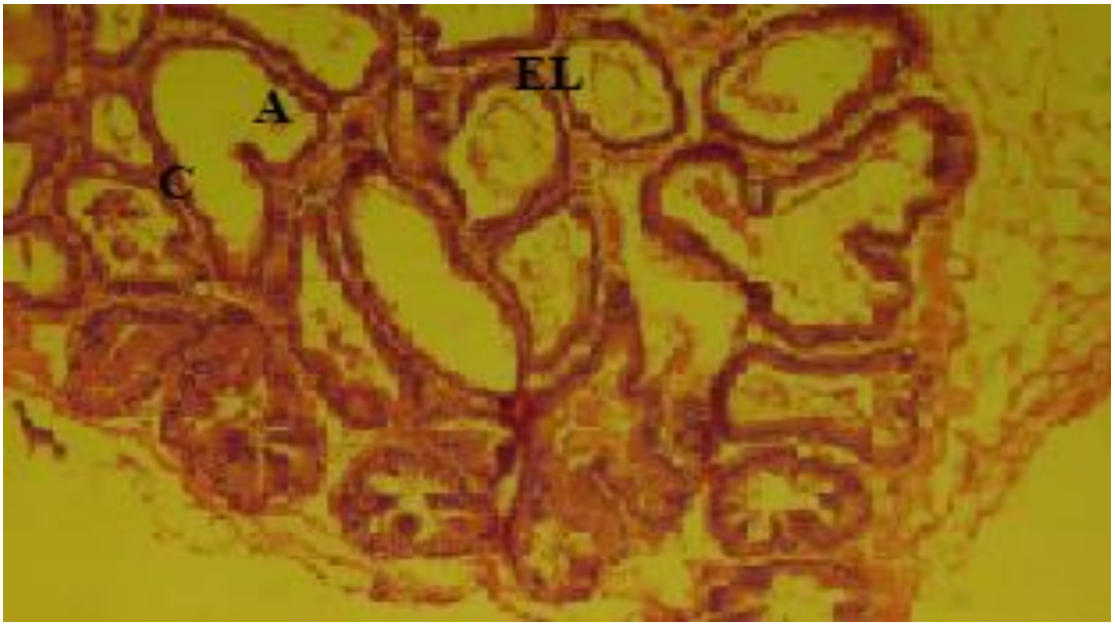

Figure 6: Micrograph of prostate tissue from the negative control group. Showed granular folds/epithelial projections causing narrowed lumen. Degeneration of the epithelial cells observed. The lumen is smaller and irregular. Excess growth of connective tissue (C) was also observed. Acini (A) appeared irregular.(x40)

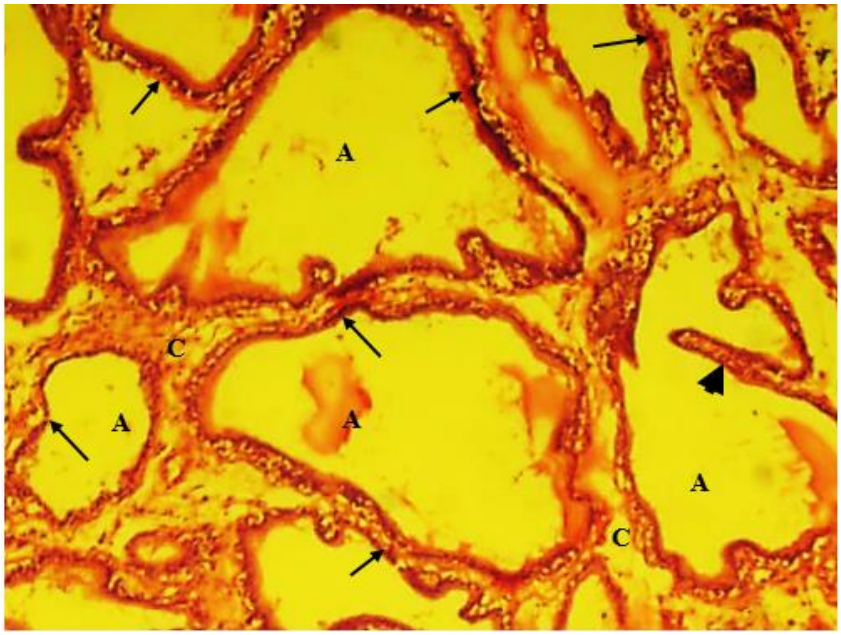

Figure 7: Micrograph of prostate tissue of rats in group 3- administered 25 mg/kg of Fraction A. Glandular fold was present as shown by arrows in (A) resulting in narrowed lumen but were reduced. The Acini(A) showed increased lumen area and less irregularity. The connective tissue (C) appeared normal and less thickened. Thickness of the epithelial lining (EL) appeared less degenerated. (X 40)

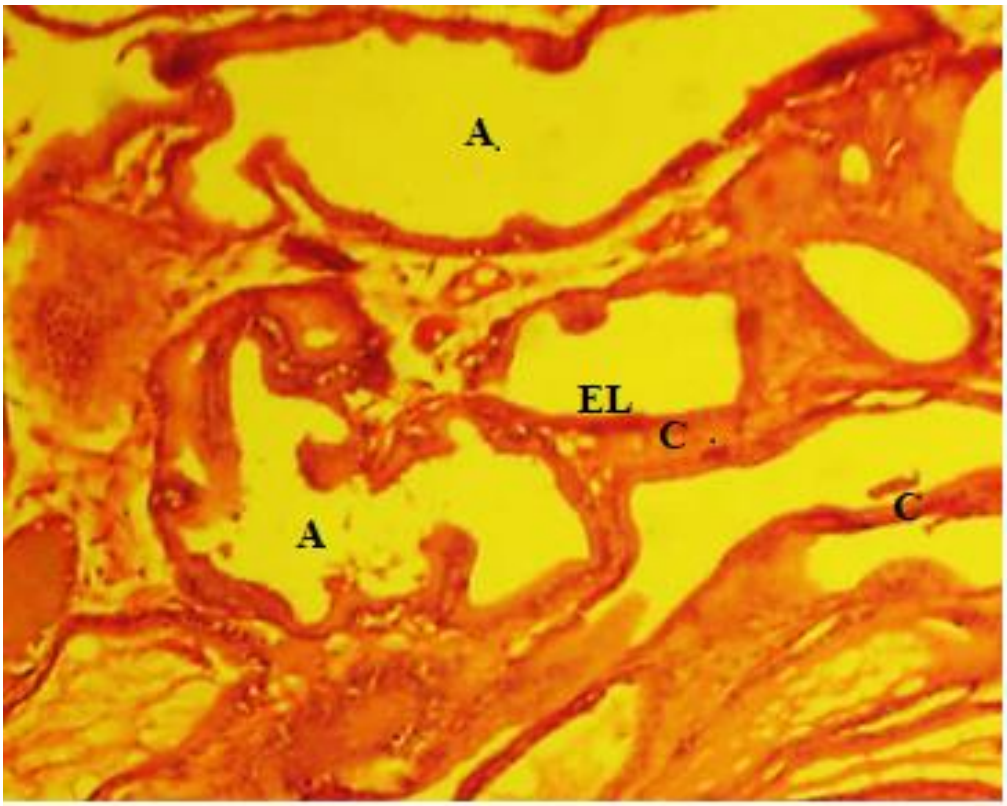

Figure 8: Micrograph of prostatic tissue of group 4 administered 50mg/kg of Fraction A. Growth of connective tissue (C) observed. Glandular projections seen, narrowed lumen and also thickness of the Epithelial Lining (EL). Shape of the Acinus (A) appears irregular.(x40)

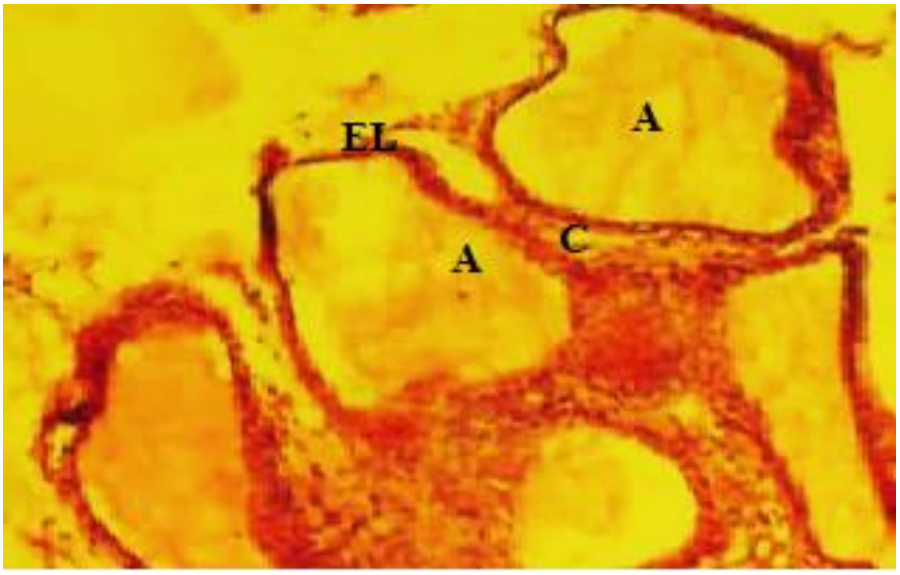

Figure 9: Micrograph of prostate tissue of animal in the positive control (Reference) group. Glandular folds not visible. Acini (A) are still irregular but slightly dilated with wider lumina. Epithelial lining (EL) less thickened and connective tissue (C) near normal.(x100).

3.4. Effect of Fraction A of the aqueous extract of *A.djalonensis* on serum testosterone level

No difference was observed with the serum testosterone levels across all groups. Graph illustrated in Figure 10 also shows that no statistically significant difference was observed at $P<0.05$.

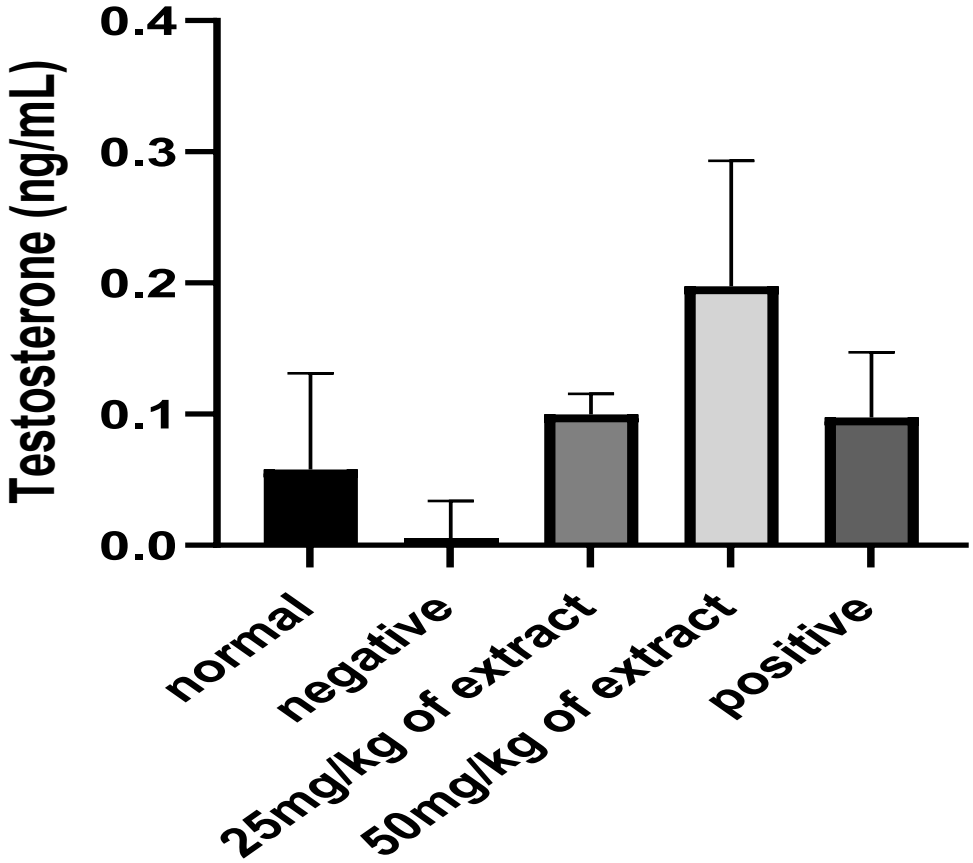

Figure 10: Effects of Fraction A on serum Testosterone level

3.5. Effect of Fraction A of the acqueos extract of *A.djalonensis* on serum Prostate Specific Antigen (PSA) level

No difference was observed with the serum PSA level across all groups. Graph illustrated in Figure 11 also shows that no statistically significant difference was observed at $P<0.05$.

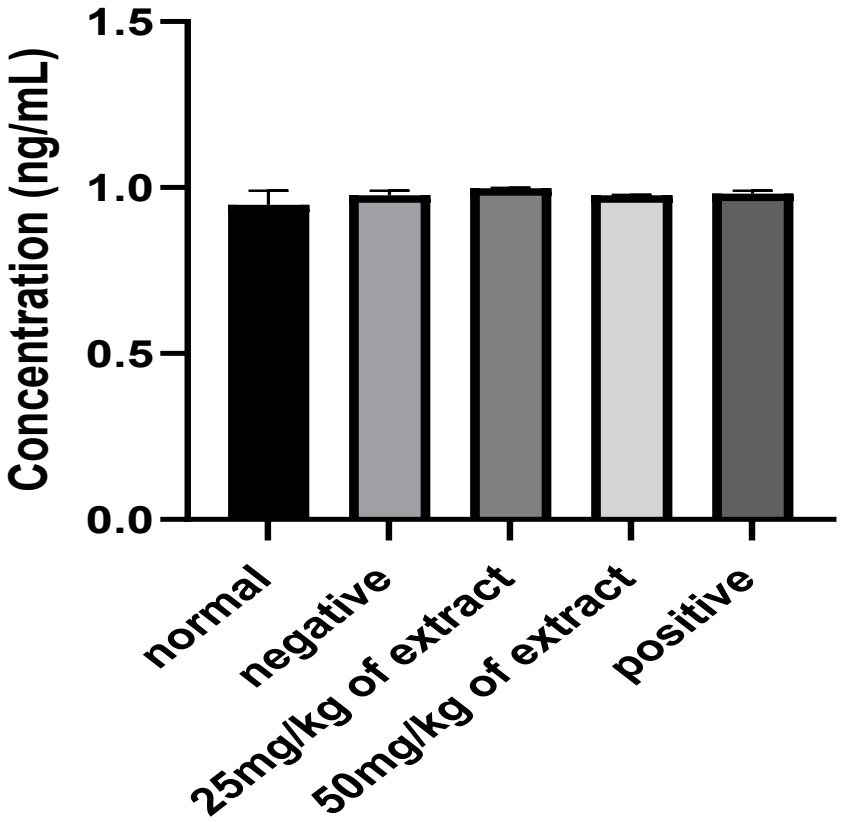

Figure 11: Effects of fraction A on serum PSA level

CHAPTER 4

4.0 DISCUSSION AND CONCLUSION

4.1 DISCUSSION

Benign prostate hyperplasia (BPH) is a non- malignant hyperplasia of the prostate tissue usually common among older men. The onset of BPH occurs in the TZ (Translational Zone) of the prostate, and due to its unique location, it can be exposed to potential irritants and after years of exposure to irritants and urinary toxins, they could disrupt tissue homeostasis and cause damage to the cells, subsequently, the disease is progressed by dihydrotestosterone which stimulates the growth of cells and ultimately a cascade of chronic inflammation within the tissues of the prostate (Xu.G *et al*., 2024). These combined effects cause obstruction of the urethra and bladder outflow leading to clinical manifestation of Lower Urinary Tract symptoms and incomplete emptying of the bladder seen in patients with Benign Prostatic Hyperplasia.

BPH is usually managed, however, the cost of conventional medicines and the side effects associated with these medicines causes a shift towards the use of CAM for the management of BPH (Stewart *et al*., 2023). One of these plants used traditionally for the management of BPH is *Anthocleista djalonensis.* (Adebayo et all 2022).

In this study, the anti-BPH activity of *A.djalonensis* is explored. The identification of its possible biologically active component followed a systematic and step-wise Chromatographic strategy. Vacuum Liquid Chromatography (VLC) was first carried out on the aqueous fraction of the leaves of the plant this separated the fractions based on their solubility in the eluting solvents after which they were pooled together based on their similar Thin Layer Chromatography (TLC) profiles. The essence of doing the VLC is it narrows the constituents present, reducing sample complexity. After this, column Chromatography was performed on the VLC fractions allowing further purification;

its mechanism of separation is by adsorption (Ishita Vij, 2023) with the silica stationary phase yielding different fractions that were subjected to TLC. The purpose of TLC was to identify fractions containing similar constituents. After these, Fraction A was afforded. This sequential approach ensures progressive purification from bulk simplification (VLC) to fine resolution (CC) to analytical confirmation (TLC). Although phytochemical screening was not performed in this study, these purification steps enrich moderately polar molecules with constituents such as iridoids, xanthones, saponins, tannins, phenol and alkaloids some of which have been attributed with anti oxidant, anti inflammatory, reported to be relevant in BPH (Elsherbini *et al.,* 2022; Okunrobo *et al.,*2008; Enoghase *et al.,* 2025; Adebayo *et al.,* 2022). The effect of afforded fraction A on testosterone-induced benign prostatic hyperplasia in male Wistar rats is explored in this study.

One of the ways used to assess the anti-BPH activity of fraction A is using Prostatic indices that include: Prostate weight, Prostate Volume and, Prostate Index. Prostatic parameters are a set of measurements taken from the prostate gland to confirm disease induction, BPH in this case, and to quantify the effect of a treatment, in this case Fraction A of the leaves of *Anthocleista djalonensis.* Changes in the prostate indices have been used to monitor the effects of drugs in BPH (Ihejieto *et al.,* 2024) The reduction in these indices in Fraction A as seen in Table 2, showed its activity. Prostatic Index (PI) is calculated by dividing the prostate weight by body weight. P.I normalises the prostate weight to the individual body weight by this, it eliminates the bias of individual body size variation although its clinical relevance has not been fully established. Increased body mass index and body fat has been identified as a risk factor for developing BPH (Ofeimun *et al.,* 2021). The prostate index for animals in the negative control group was observed to be higher compared to that of the 50 mg/kg of Fraction A, 25 mg/kg of Fraction A, positive

control and normal control group and showed a statistically significant value of $p<0.001$ using one way Analysis of Variance (ANOVA). And post-hoc analysis showed $p<0.001$ for the Negative control group versus other groups as represented in Figure 4. This shows that Fraction A causes a statistically significant reduction in prostate index compared to the untreated animal, confirming its Anti- BPH activity; this could be due to the fact that it interferes with the pathological growth process driven by testosterone leading to a measurable reduction in prostate enlargement. There was no statistical significant difference between Fraction A and finasteride, which could mean that Fraction A may be similar to the reference drug, Finasteride. No statistically significant difference was also observed between the normal control group and the group administered 50 mg/kg of the extract, which could mean that the extract showed reversal to a near physiologic state, however a statistical significant difference was observed between the group given 25mg/kg of the extract and the normal control group, this could mean that while the 25 mg/kg of the extract may reduce Hyperplasia, it may not be full reversal to normal physiologic states

Increase in the weight of the prostate as seen in the negative control group significantly shows prostatic hyperplasia also seen in *O.Majorana* study by Elsherbini *et al.,* 2022. Prostatic hyperplasia is usually due to proliferation of stroma and epithelial cells of the prostate (Michael *et al* 2024) this results in increased weight of the prostate. The prostate weight also showed similar results with the Prostate Index, however, there was no statistically significant difference between 25 mg/kg of Fraction A and the normal group as shown in Figure 3.

For the prostate Volume, One Way-Anova showed that there was a statistically significant difference at $P<0.001$ and showed similar results for the post-hoc analysis as seen with the prostate weight. However, further studies have to be carried out to establish these claims.

Another important tool used for evaluating and quantifying the effects of an intervention on disease progression is histological examination of tissues, in this study, the prostate tissue. (Ihejiet *et al.,* 2024). The normal control group (Figure 5) which serves as the baseline showed distinct, well-formed Acini with a wide clear lumen. Epithelial lining is normal, and connective tissue is also normal and minimal indicating non-pathologic state. In contrast, the negative control group (Figure 6) exhibited microscopic features of hyperplasia. Epithelial cells showed degeneration and granular folds which narrow the lumen were observed, and excess growth of connective tissue. This confirms that induction with testosterone produced glandular Hyperplasia seen as excess epithelial folding and stroma hyperplasia seen, as excess connective tissue as seen in BPH (Michael *et al*., 2024)

Groups administered Fraction A displayed feature of lesser hyperplasia in prostate tissue compared to as seen in the negative control group supporting previous findings from the prostatic parameters. At 25mg/kg, the prostate tissue showed reduction in glandular folds leading to increased lumen area and also showed less thickening of the connective tissue. This normalisation of the stroma component is recognised as a target for plants. (H.A..Kenny *et al*., 2021). However, a paradoxical result was observed where the 50mg/kg group showed more similarity with the negative control group compared to the 25mg/kg group, a reduction in therapeutic effect at higher doses suggests a non-linear pattern, consistent with hormesis, a phenomenon that has been seen with some natural phytochemicals. This could be due to saturation of receptor, or compensatory feedback inhibition (Jadwiga et al., 2020) as fibrosis is seen in the group given 50mg/kg of Fraction A. Fibrosis observed could be a side effect of high dose, however further study should be carried out to ascertain these claims.

As seen in Figure 9, the positive control group, glandular folds were not visible, the epithelial lining were less, this validated finasteride mechanism of reversing glandular Hyperplasia by blocking the production of dihydrotestosterone which causes the epithelial cells of the prostate to shrink. (Chislet *et al.,* 2023).

Testosterone is a prohormone converted to dihydrotstosterone by 5 alpha-reductase. In BPH, testosterone play a key role due to its being converted to dihydrotstosterone which ultimately causes proliferation of cells in prostatic tissue. Finasteride, the reference drug in this study and a conventional drug, acts by inhibiting the action of 5-alpha reductase. As earlier discussed, progression of BPH depends on the synthesis of testosterone (Ishola et al.,2023). However, in this study, no statistically significant difference was observed between the control groups and that of Fraction A. this result is parallel to that observed by Okafor et al 2025 amongst other previous studies. This could be as a result of individual variability and peculiarity of the animals hence further studies have to be carried out by using more animals more than that used in this study and for a longer period. Another reason could be the storage of the serum. Proposed reasons for this could most significantly include the fact that in this study, serum testosterone was measured, however, BPH is progressed by intraprostatic dihydrotestosterone, which could not have been found in the serum due to the short duration of the study.

Given that it has been reported in previous studies of the anti-inflammatory effect of *A.djalonensis* by Enoghase *et al*.,2025, this could propose a mechanism of action for this plant which could be by suppressing pro inflammatory cytokines which are usually upregulated in BPH. The study by Samuel et al also revealed the antiproliferative effect of this plant, hence this plant might exhibit anti-BPH effect by inhibiting the proliferation of cells that showed hyperplasia. However, more study needs to be carried out since it exhibited unusual dose response curve for some parameters.

In this study, mortality was recorded, hence, further study has to be carried out to determine the level of toxicity that may not be appropriate to BPH.

A marked reduction in weight was also observed in the animals after induction and on the final day, since there is no evidence supporting that BPH causes weight loss, other than

weight gain being a risk factor, one can say that the causes of the weight reduction could be due to metabolic illnesses which further necessitates the need for toxicity studies to be carried out on the plant extract.

4.2 CONCLUSION

From the results gotten from the prostate index, prostate weight and prostate volume reduction, and also from histological findings, it can be concluded that the plant possess effect against Benign Prostatic Hyperplasia, however, the results from the serum testosterone and PSA profile does not support this, hence, further research has to be carried out on the toxicity levels and its mechanism of action(s).

4.3 RECOMMENDATION

These recommendations are made in the event of Future research for the study of *A.djalonensis* on Benign Prostatic Hyperplasia.

A. Further research to the toxicity levels of the plant
B. More animals should be used for further studies and for a longer time period.
C. More comprehensive screening should be carried out to identify and isolate the exact bioactive compounds responsible for its anti-BPH activity.
D. Further studies should be carried out to investigate the particular mechanism of action.

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