EFFECT OF *ALLIUM CEPA* BULB AND *ANNONA MURICATA* PULP JUICES ON TESTOSTERONE AND OESTRADIOL-INDUCED BENIGN PROSTATIC HYPERPLASIA IN ALBINO RATS

BY

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CERTIFICATION

This is to certify that this work "Effect of *Allium cepa* Bulb and *Annona muricata* Pulp Juices on Testosterone and Oestradiol-induced Benign Prostatic Hyperplasia in Albino Rats", was carried out by Ihejieto, Hope Amarachi (20194190038), in partial fulfilment of the requirements for the award of the Degree of Doctor of Philosophy (Ph.D) in Industrial Biochemistry in the Department of Biochemistry of the Federal University of Technology, Owerri.

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DEDICATION

This thesis is dedicated to the Holy Trinity; God the Father, the Son and the Holy Spirit, and to my husband, Chinedu, and my lovely children, Kosisochukwu, Chinedu and Nkemjika.

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ABSTRACT

Benign prostatic hyperplasia (BPH) is an age-related condition accompanied by lower urinary tract symptoms (LUTS). Management with conventional therapy is the only available solution, with its attendant side effects. This study investigated the effect of *Annona muricata* (soursop) pulp and *Allium cepa* (onion) bulb juices on testosterone and oestradiol-induced BPH in albino rats. The two plant samples were assessed for *in vitro* antioxidant properties, and acute toxicity studies. A total of 54 albino rats were used in the *in vivo* study, randomly distributed into 9 groups (A to I) of 6 rats each. Groups B to I were induced with 10 mg/kg b.w of 1:1 testosterone and oestradiol every two days. They were treated as follows: Groups A (normal control), B (disease control), C (standard control), D (10 ml/kg b.w soursop), E (10 ml/kg b.w onion), F (10 ml/kg b.w 1:1 soursop and onion), G (20 ml/kg b.w soursop), H (20 ml/kg b.w onion), and I (20 ml/kg b.w 1:1 soursop and onion), for 28 days. The animals were weighed weekly, and were sacrificed at the end of the treatment. Blood samples and prostates were collected for relevant assays. The results showed that soursop pulp and onion bulb juices possessed antioxidant properties, and LD_{50} above 5,000 mg/kg b.w. The test samples also ameliorated BPH development in varying degrees. This was evident in their ability to maintain body weight, reduce prostate weight, improve *in vivo* antioxidant status, hormonal and PSA levels, and elicited anti-inflammatory, antiproliferative and pro-apoptotic responses. Considering the values obtained from the test groups, it can be adduced that soursop juice had better potency, especially, at low dose. The results of histological evaluation of the prostates corroborates this finding. Soursop juice was then packaged. Preliminary sensory evaluation and microbial analysis showed that the soursop juice was acceptable and safe for consumption. Soursop therefore is a beneficial fruit and its consumption should be encouraged, especially by older men.

Keywords: *Allium cepa* bulb juice, *Annona muricata* pulp juice, Testosterone and Oestradiol-induced, Benign prostatic hyperplasia.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Benign prostatic hyperplasia (BPH), also referred to as benign prostatic hypertrophy (McVary, 2020) or enlarged prostate (in simple term), is a disease condition that affects aging men. It is a progressive condition identified by enlargement of the prostate that is accompanied by lower urinary tract symptoms (LUTS) (Emberton et al., 2008). It represents an inevitable phenomenon among aging males and develops from the periurethral and transition zones of the prostate gland (Lepor, 2005).

The LUTS can be divided into two groups; the obstructive symptoms in voiding (terminal dribbling, hesitancy, poor stream, incomplete voiding, and overflow incontinence) and irritative or filling symptoms (dysuria and nocturia, increased urinary frequency and urgency) (Lepor, 2005; Lukacz et al., 2011). Although, BPH is not common before the age of 40, but by 50 years of age, roughly 50 % of men begin to experience BPH-related symptoms. Prevalence of BPH rises by 10 % every 10 years, reaching 80 % at about 80 years of age (Bharti, 2017). An estimated 75 % of men over the age of 50 present with symptoms that relates to BPH, and 20 to 30 % of men 80 years and above develop severe symptoms requiring treatment (John Hopkins Medicine, 2023), which may require surgery to manage if unresponsive (Bortnick, Brown, Simma-Chiang & Kaplan, 2020).

The pathophysiology of BPH remains unclear despite numerous studies (Dobrek & Thor, 2015). The development and progression of BPH appear to be mediated by a number of mechanisms. Although, in addition to the central role of aging in BPH, studies also identified the crucial role of inflammation, metabolic syndrome, and hormonal alterations (Berger et al., 2005).

The majority of the medicaments used in treating BPH are α-adrenoreceptor antagonists (α-blockers) and 5 α-reductase inhibitors. The alpha-blockers work by relaxing prostate and bladder neck muscles (Fine & Ginsberg, 2008), which reduces urine flow obstruction, thus, improving the rate of urine flow, while 5 α-reductase inhibitors stop further enlargement or induce shrinking of prostate by inhibiting conversion of testosterone to dihydrotestosterone (Kim, Brookman & Andriole, 2018). These drugs have side effects such as, dizziness, fainting, headaches, light-headedness, low blood pressure and sometimes, retrograde ejaculation by the

α-blockers (Unal, Micoogullari, Okulu & Kayigil, 2020). Similarly, low libido, erectile dysfunction, and retrograde ejaculation have been associated with the 5 α-reductase inhibitors (Kim et al., 2018).

All over the world, there is a growing trend towards the use of complementary and alternative medicine (CAM) for a vast number of illnesses. Medicinal plants are a major source of CAM for various ailments. There is a growing interest in the development of phytotherapeutic agents, due to the widely held belief that they are more cost-effective, safer and have less side effects than conventional therapy for the management of diseases (Calixto, 2000; Thompson et al., 2003).

Allium cepa Linn (common name - onion) is the most frequently cultivated species of the genus *Allium* found in Africa, Asia, Europe and North America (Chakraborty et al., 2022). With present global production of about 44 million tonnes, it is the second most significant horticultural crop after tomatoes. Onion is an ingredient regularly included in many dishes. It is increasingly consumed due to its health benefits and flavour. Onion contains, in abundance, alk(en)yl cysteine sulphoxides (ACSOs) and flavonoids, perceived to be of benefit to human health (Ranjith & Vinoth, 2022). Anthocyanins and flavonols, such as quercertin and its derivatives, are flavonoids subgroups found in onions (Benitez et al., 2011). Compounds from onions have been reported to have anicarcinogenic, antiplatelet, antithrombotic, antibiotic and antiasthmatic effects (Griffiths, Trueman, Crowther, & Thomas, 2002).

Annona muricata L. (commonly referred to as soursop or graviola) is a member of the family Annonaceae which comprises of about 130 genera and 2300 species (Mishra, Ahmad, Kumar & Sharma, 2013). It is native to the warmest tropical parts of the North and South America, and now widely dispersed throughout the subtropical and tropical regions of the world, including Malaysia, India and Nigeria (Adewole & Caxton-Martins, 2009). *Annona muricata* is an erect, evergreen, terrestrial plant reaching 5 to 8 m in height, with an open roundish canopy with glossy, dark green leaves. The edible fruits of this plant are heart-shaped, large, and green in colour, with the diameter ranging from 15 to 20 cm (Ribeiro de Souza, da Silva, Afonso & Scarminio, 2009).

In traditional medicine, all the parts of *Annona muricata* tree are widely used against an array of human diseases. The fruit is used as natural remedy for arthritic pain, diarrhoea, neuralgia, fever, arthritis, dysentery, rheumatism, skin rashes and malaria, and it is also consumed by lactating mothers to boost breastmilk production after childbirth (Hadju & Hohmann, 2012;

Moghadamtousi et al., 2015). The leaves are utilized in treating headaches, diabetes, insomnia and cystitis (Wele et al., 2004; Adewole & Caxton-Martins, 2006; Mishra et al., 2013). Furthermore, internal administration of the decoction of soursop leaves is thought to exhibit neuralgic and anti-rheumatic effects (Adewole & Caxton-Martins, 2009). It is believed that the crushed seeds possess anthelmintic activities against internal and external parasites and worms (Moghadamtousi et al., 2015).

1.2 Statement of the Problem

More than 42 % of men between the ages 51 to 60, 70 % of men between 61 to 70 years of age and up to 90 % of men from age 80 upwards are affected by BPH. Prostate enlargement often results in LUTS, and this negatively impacts the quality of life of the patients (Emberton et al., 2008). There is no known medical cure yet for BPH. The primary goal of BPH management has been to treat and alleviate the symptoms of the condition (Lerner et al., 2021). However, it is very common for the condition to reoccur in the patients once treated. Conventional BPH treatment regimens in use today have produced some adverse side effects. Asides being expensive, and causing erectile dysfunction and urinary incontinence, other side effects of conventional therapy include toxicity and growth inhibition to normal cells. Hence, there is a pressing need to discover and develop phytotherapeutic agents with curative effect on BPH and related diseases.

1.3 Aim of the Study

The aim of the study was to determine the effect of *Allium cepa* (onion) bulb and *Annona muricata* (soursop) pulp juices on testosterone and oestradiol-induced benign prostatic hyperplasia in albino rats.

1.4 Objectives of the Study

The specific objectives of this study include, to;

1. Determine the acute toxicity (LD_{50}) level of *Allium cepa* bulb and *Annona muricata* pulp juices.
2. Determine the *in vitro* antioxidant properties of *Allium cepa* bulb and *Annona muricata* pulp juices.

3. Study the body weight changes, prostate weight (PW), prostatic index (PI), relative prostate weight (RPW) and percentage prostate increase inhibition (PPII) of the albino rats.
4. Determine the effect of the juices on oxidative stress markers such as superoxide dismutase (SOD), glutathione peroxidase (GPx), Glutathione-S-transferase (GST), and catalase (CAT) activities, malondialdehyde (MDA), reduced glutathione (GSH) level, total protein, as well as, protein carbonyl (PCO) groups concentrations of the albino rats.
5. Examine the effect of *Allium cepa* bulb and *Annona muricata* pulp juices on the histopathology of the prostate gland of the albino rats.
6. Ascertain the effect of *Allium cepa* bulb and *Annona muricata* pulp juices on serum levels of testosterone, dihydrotestosterone (DHT), prolactin, prostate specific antigen (PSA), and prostatic concentrations of cytokines, such as, interleukin- (IL-) 8, tumor necrosis factor (TNF)-α and lymphotoxin-α (TNF-β) of the albino rats.
7. Assess the effect of the juices on the expression of proliferating cell nuclear antigen (PCNA) and Caspase-3 activity, and the activity of 5-α reductase enzyme *in vivo* and *in vitro*.
8. Package the most effective juice extract in presentation form.

1.5 Justification of Study

Despite the high health concerns surrounding BPH, its pathophysiology is still unclear (Dobrek & Thor, 2015). A number of mechanisms appear to be involved in the onset and progression of BPH. Aging plays a central role, as well as inflammation, metabolic syndrome, and hormonal alterations (Berger et al., 2005). Conventional medicines currently used in treating BPH cause adverse side effects (Unal et al., 2020; Kim et al., 2018).

Medicinal plant therapy is a relatively cheaper alternative for its users, and are apparently considered relatively safer. Phytomedicine is generating an increase in interest and use. It is estimated that 75 to 80 % of the world's population access medicinal plants as the foremost treatment option (Ekor, 2013). However, studies have shown that *Allium cepa* and *Annona muricata* possess pharmacological properties with beneficial effects, which includes, antitumor, antifungal, antithrombotic, antibacterial, hypocholesterolemic and anti-inflammatory, antiprotozoal, and anticancer effects (Matsuura, 2001; Lanzotti, 2006; Gyesi, Opoku & Borquaye, 2019). In view of the aforementioned properties of the plants, *Allium cepa* and *Annona muricata* therefore has the potential for their use as treatment for BPH.

1.6 Scope of the Study

This study was limited to preparation of *Allium cepa* bulb and *Annona muricata* pulp juices and determining their effects on testosterone and oestradiol-induced BPH in albino rats. Standard laboratory techniques were employed in the acute toxicity studies, *in vitro* antioxidant properties assays, and *in vivo* studies. The *in vivo* studies included determination of body weight changes, prostate weight (PW), prostatic index (PI), percentage prostate increase inhibition (PPII) and relative prostate weight (RPW) of the albino rats, effects on oxidative stress markers, and histopathology of the prostate gland of the albino rats. Other assays carried out included serum hormone levels, PSA, and prostate concentrations of cytokines, expression of PCNA, Caspase-3 activity, and 5-α reductase enzyme activity *in vivo* and *in vitro*. The most effective juice extract was also packaged.

1.7 Significance of the Study

There is currently a great deal of interest in developing phytotherapeutic agents for BPH management as they are perceived to be safer, more cost-effective and have lesser side effects than their conventional/orthodox alternatives. The increase in awareness being given is fostering the increase in demand for herbal therapies. As more people shy away from conventional/orthodox medicines for the treatment of conditions encompassing sexual function and fertility, as well as BPH, medicinal plant therapy presents itself as an indispensable resort. Therapeutic agents with the ability to influence apoptosis have emerged as potential targets for optimizing treatment of BPH and its related cancers.

CHAPTER TWO

LITERATURE REVIEW

2.1 Accessory Glands of the Urino-Reproductive System in Human Males

The prostate

The prostate is a small exocrine gland regulated by androgens, measuring 3 cm long, about 4 cm wide and 2 cm in depth. It is situated at the neck of the bladder where it connects the *ductus deferens* at the ejaculatory duct (Taylor, 2021). It is anterior to the rectum, surrounding the beginning of the urethra. It is mostly made up of fibromuscular tissue and exocrine glandular tissue. The dense irregular connective tissue and smooth muscle tissue are the two kinds of tissue that make up the fibromuscular tissue (Davidoff, 2021). The connective tissue comprises of numerous collagen fibres that cover the outermost layer of the prostate and the urethra. The prostate is typically partitioned into zones and lobes. The lower capsular area of the posterior segment of the prostate gland is the peripheral zone (PZ). This includes the distal urethra and it is also the location of origin for roughly 70 to 80 % of all prostate cancers (PCas). The central zone (CZ) is the zone surrounding the ejaculatory ducts and about 2.5 % of PCas occur here. The transition zone (TZ) is the site of origin for about 10 to 20 % of PCas. It encompasses the proximal urethra and is the region of the prostate gland that continues to grow throughout the lifetime of a man. It is also the main region where BPH occurs (Paolone, 2010).

Seminal vesicles

The seminal vesicles (SV) are a pair of folded and coiled tubular glands lateral to the *ductus deferens* and postero-inferior to the bladder. The SV has a thick muscular wall, a mucosa made up of a lining of interspersed columnar cells and a laminar propria. The lumen is where the secretions of the vesicular glands are stored (Young, Lowe, Stevens & Heath, 2006). The seminal vesicle epithelia are made up of majorly stratified columnar cells whose activity and height is dependent on plasma testosterone.

The testes

The testes are a pair of oval-shaped organs located in the scrotal sac responsible for the production of the sperms and androgens. The testis spreads into lobules that contain the seminiferous tubules (tightly coiled tubings). The germ cells and Sertoli cells are in the seminiferous tubules, while the Leydig cells are around it. The germ cells differentiate into the

sperm cells, the Leydig cells synthesize the testosterone, while the Sertoli cells protect and support the sperms (Walker & Cheng, 2005). Through the *ductus deferens*, the sperms are conveyed towards the prostate and the urethra via the spermatic cord.

2.2 Hormones

Hormones are chemical messengers that regulate body functions. They bind to specific receptors to activate biochemical responses after being secreted. Depending on their structural characteristics, they are divided into the following types:

1. Peptide hormones: These are polypeptides (consisting of amino acids). They mediate processes such as metabolism.
2. Amino acid derivatives: These are hormones derived from amino acids, as the name implies, and are relatively small molecules. Some are water soluble (e.g, epinephrine) while some are insoluble in water (e.g, thyroxine).
3. Steroid hormones: These are cholesterol-derived molecules, and are classified into two categories:
   i. Corticosteroids, which are produced in the adrenal cortex.
   ii. Sex steroids, which are produced in the testes and ovaries (the androgens, oestrogens, progesterone) (Molnar & Gair, 2015).

Androgens

Androgens are the male sex hormones synthesized in the testes in males and, to a lesser extent, in the adrenal cortex. They are primarily involved in the development of male specific phenotype and reproduction (Kim, 2008). The androgens include testosterone and dihydrotestosterone.

1. Testosterone: This is a steroid hormone, and the major circulating androgen in men, synthesized in the Leydig cells of testes. It plays an important role in regulation of male sexual differentiation, spermatogenesis and male secondary characteristics expression, such as body hair growth, and increased bone and muscle mass. The Leydig cells synthesize majority (about 95 %) of the testosterone in males regulated and controlled mainly by luteinizing hormone (LH) (Aladamat & Tadi, 2022), while the remaining small fraction is synthesized in the adrenal cortex through the action of adrenocorticotropic hormone (ACTH) (Turcu & Auchus, 2015).

   Testosterone synthesis (Figure 2.1) begins with the conversion of cholesterol to pregnenolone in the inner mitochondrial membrane of the Leydig cells. The hypothalamus

secretes gonadotropin releasing hormone (GnRH) which stimulates the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in the anterior pituitary. LH and FSH are two gonadotropic hormones that bind to their specific receptors in the gonads. While the FSH is involved in spermatogenesis, the LH binds to the luteinizing hormone/choriogonadotropin receptor (LHCGR) causing an increase in phosphorylation of steroidogenic acute regulatory (StAR) protein which then regulates the cholesterol transfer to the inner mitochondrial membrane. Then, the cholesterol side chain cleavage enzyme, CYP11A1 (P450scc) converts cholesterol to pregnenolone (Miller, 2002). The pregnenolone passively diffuses out of the mitochondria, and the subsequent steps then occur in the smooth endoplasmic reticulum.

In humans, the Δ5 (delta 5) pathway is favoured, where the pregnenolone is converted to 17 OH-pregnenolone by the 17 alpha hydrolase activity of CYP17A1 (Miller, 2002; Flück, Miller & Auchus, 2003). The 17 OH-pregnenolone is then converted to dehydroepiandrosterone (DHEA) by the 17,20 lyase activity of the CYP17A1. The HSD17B3 (17-beta-hydroxysteroid dehydrogenase), which is exclusively expressed in the testes, then converts DHEA to androstenediol or androstenedione and then into testosterone (Lawrence, O'Donnell, Smith & Rebourcet, 2022). Testosterone gets converted to dihydrotestosterone by the enzyme 5 α-reductase.

When blood testosterone levels are high, testosterone inhibits its own secretion by negative feedback to the hypothalamus which then suppresses GnRH secretion, and also to the anterior pituitary reducing its response to GnRH stimuli. (Plant & Marshall, 2001). Increase in LH and FSH results in rise in production of steroids (17-hydroxyprogesterone, DHEA, testosterone, LH, and FSH) in the gonads. FSH increases the conversion of androgens to oestrogens by upregulating aromatase (Clark, Prough & Klinge, 2018). Testosterone can also be metabolized into oestradiol-17β (the potent oestrogen), by aromatase (CYP19; Cytochrome P450 19). The growth and differentiation of prostate is also directly and indirectly affected by oestrogens (Nicholson & Ricke, 2011).

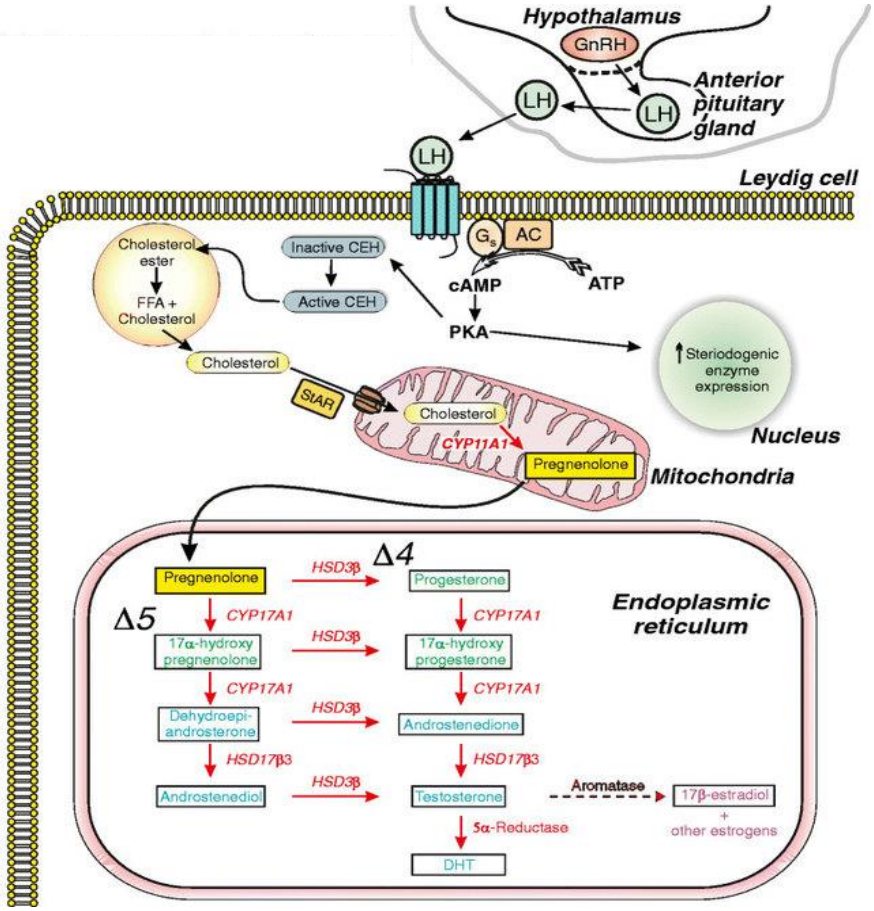

Figure 2.1: Schematic representation of major pathways for testosterone biosynthesis in the gonads (Ayaz & Howlett, 2015).

2. Dihydrotestosterone: The 5-α dihydrotestosterone (DHT) (Figure 2.2) is the product of the metabolism of testosterone. It is the most potent prostatic androgen and also the primary androgen responsible for the growth, development, maturation and function of the prostate. It is formed by the reduction of testosterone by 5-α reductase in the peripheral tissue of the prostate (Tuck & Francis, 2009). It has also been observed to regulate the expression/activity of insulin-like growth factor 1 (IGF-I), epidermal growth factor (EGF), and fibroblast growth factor (FGF)-related proteins like keratinocyte growth factor (KGF), and their receptors (Carson & Rittmaster, 2003).

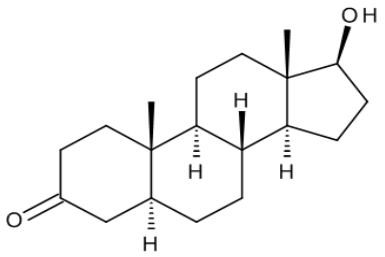

Figure 2.2: Structure of dihydrotestosterone (Tuck & Francis, 2009).

2.3 Diseases of the Prostate Gland

The prostate gland is affected by a number of clinical conditions. These include prostatism, prostalgia, prostatitis, BPH and prostate cancers (John Hopkins Medicine, 2023).

1. **Prostatism**

Prostatism is any disorder of the prostate that affects the flow of urine from the bladder. Hence, the symptom of prostatism is decrease in the force of urinary flow as a result of obstruction of the bladder neck. BPH is the most common cause of prostatism.

2. **Prostatitis**

Prostatitis describes the inflammation or infection of the prostate gland. Fever, pain, discomfort, and frequent or infrequent urination may be present. Prostatitis mainly occurs in men younger than 50 years.

3. **Prostatalgia**

Prostatalgia, also referred to as prostatodynia, is a condition characterized by pain in the prostate gland. It is frequently a symptom of prostatitis (John Hopkins Medicine, 2023).

4. **Prostate cancer**

Any cancer that develops within the prostate is known as prostate cancer (PCa). It is arguably the prostate condition of utmost concern.

5. **Benign prostatic hyperplasia (BPH)**

Benign prostatic hyperplasia (BPH), also known as nodular hyperplasia of the prostate. It is the most prevalent benign prostate problem, and poses a greater health risk than PCa. This is because many more men with indolent prostate cancer die with the condition than from it (Smith, Dunn, Strawderman & Pienta, 2012).

It is characterized by increase in the size of the epithelial and stromal cells of the prostate gland and compression of the urethra that results from the abnormal proliferation of periurethral cells. This involves formation of discrete nodules in the periurethral region which compresses the urethra causing a decrease in the normal urine flow. In most cases, the stromal cells are affected. The posterior urethral glands (PUG), the transitional zone (TZ), and to a lesser extent, the peripheral zone (PZ) are the main sites affected, as well as, the glandular epithelial cells. In 1990, John McNeal proposed that in the early stages of embryonic re-awakening, the growth of the number of the small stromal periurethral and TZ glandular nodules is reinitiated (Brennen & Isaacs, 2018). This is followed by a phase characterized by an increase in the numbers of larger nodules and a rise in the stromal-epithelial ratio. BPH is often characterized by urinary retention, urinary frequency, and other urinary symptoms termed LUTS and may be classically defined as the histological development of prostatic hyperplasia.

2.3.1 Causes of BPH

1. Hormonal and growth factors

Dihydrotestosterone (DHT), the product of the reduction of testosterone by 5-α reductase enzyme in the prostate, is the principal androgen that influences prostate volume. Both androgens; testosterone and DHT, promote prostate cell proliferation (Feldman & Feldman, 2001), while oestrogen regulates the development of BPH indirectly, by the local conversion of androgens to oestrogen (Ho, Nanda, Chapman & Habib, 2008).

The underlying cause of BPH development is not entirely clear at the molecular level. However, a complex interplay of androgens, growth factors like epidermal growth factor (EGF), stromal epithelial interactions, transforming growth factor-β (TGF-β), insulin-like growth factor (IGF), neurotransmitters, keratinocyte growth factor (KGF), and oestrogen are implicated (Gillenwater, Grayhack, Howards & Michell, 2002).

Oestrogen, especially E_2 (17-β oestradiol), has been noted to be the most potent oestrogen in men, and can be a potent prostatic proliferation inducer (Nicholson & Ricke, 2011). Mainly, it originates from aromatization of testosterone, while the Leydig cells secretes about 20 % (Vermeulen, Kaufman, Goemaere & van Pottelberg, 2002). Corona et al. (2014) suggested that a rise in oestrogen level may result in BPH/LUTS progression. There are reports linking BPH to higher serum concentrations of oestrogen or oestrogen/androgen ratio (Roberts et al., 2004; Hammarsten et al., 2009).

Prolactin has also been reported to be involved in stromal cell proliferation. A study showed that androgen receptor (AR) plays a role as promoter of prostate tissue development as indicated by AR suppression in transgenic mice that developed BPH spontaneously due to overexpression of prolactin. Hence, demonstrating that prolactin signaling pathway is pivotal for stromal cell proliferation and the AR receptor has the ability to modulate epithelium-stromal cell interaction (Lai et al., 2013).

2. Lifestyle factors

Lifestyle factors that may influence prostate growth include nutrition, smoking, having multiple sex partners, exercise (Parsons, 2011), and oxidative stress (Suzuki et al., 2012). Age, obesity, androgen and functional androgen receptors, dyslipidaemia, genetics, and diabetes are grouped as risk factors for BPH while basal metabolic index (BMI), high blood pressure, diet, smoking, and sexual mal-function are listed as possible risk factors.

2.3.2 Diagnosis of BPH

1. Digital rectal examination (DRE)

DRE is a medical technique involving the insertion of the finger through the rectum to inspect the prostate for abnormalities. The prostate is examined to determine if it is larger than normal for a certain age and felt for any hard or irregular areas. With a sensitivity of about 90 %, DRE can also detect cancer that has progressed past early stage (Hsu, Jonlau, Oyen, Roskams & Poppel, 2006).

3. Biochemical markers

Elevated levels of PSA has been observed to be an indicator of BPH and may result in urinary problems but not cancer. However, in 10 to 30 % of cases, BPH and occult PCa can co-exist (Alcaraz, Hammerer, Tubaro, Schröder & Castro, 2009). Prostate specific antigen (PSA) is a kallikrein-like serine protease that is typically synthesized by the epithelial cells of the prostate. When prostate cancer, prostatitis, benign prostatic hypertrophy (BPH), and other non-malignant disease conditions are present, serum levels of PSA may increase. The levels of PSA are frequently used as an index for detecting the onset of BPH because of the direct relationship between it and prostate volume. PSA levels also correlates significantly with prostate volume, and both are age-dependent (Putra, Hamid, Mochtar & Umbas, 2016). Long-term changes in symptoms scores and urine flow rate are seen in both PSA and prostatic volume.

2.3.3 Treatment of BPH

Most males (about 90 %) above 50 years of age with the BPH condition do not require medical or surgical intervention to treat the condition. The conventional treatment plans for BPH may involve non-interventional, invasive and chemotherapeutic procedures, depending on the severity of the condition. Watchful waiting and occasionally active surveillance are the non-interventional treatments involved (Ng & Baradhi, 2022).

2.4 Phytotherapy

Since 3000 BC, the therapeutic properties of plants have been described in ancient Egyptian and Chinese literature. However, the practice is believed to have existed before written history. Early accounts detailed how ancient Egyptians treated/managed diseases with garlic, absinth, myrrh, cassia, fennel, juniper and cumin (Pletea, n.d.). Medical systems like the Traditional Chinese Medicine and the Indian Ayurveda were established primarily as herbal-based treatment methods. Herbal medicines have long been known to be used for healing rituals by Americans and Africans (Sneader, 2005). For long, traditional medicine has been considered as a resource for the development of orthodox medications. A few examples of such medications are quinine, morphine, digoxin, atropine, codeine, reserpine, and vincristine.

2.5 *Allium cepa*

2.5.1 Description of *Allium cepa*

Allium cepa is scientifically classified as follows; Kingdom: Plantae; Division: Magnoliophyta; Class: Liliopsida; Order: Asparagales; Family: Alliaceae; Genus: *Allium*; Species: *A. cepa* (Chakraborty et al., 2022).

Plate 2.1: *Allium cepa* bulb (https://www.chefsmandala.com/archeology-fruits-vegetables-red-onion/)

Allium cepa (commonly known as onion or bulb onion) is a species of the genus *Allium* that is most widely cultivated. In Nigeria, it is known as "Albasa" by the Hausas, "Ayabosi" by the Igbos, and "Alubosa" by the Yorubas (FitNigerian, 2024). Onion (Plate 2.1) originated from central Asia and has been in cultivation for over 4 millennia, earning it a place as one of the oldest cultivated plants (Corzo-Martinez & Villamiel, 2012). It is a perennial herb and an underground stem. The common onion has one or two leafless flower stalks that reaches 75 to 180 cm (2.5 to 6 feet) in height. Most onions cultivated for commercial purpose are propagated using the thin, dark seeds of the plant. All over the world, the species of the genus *Allium*, including onion, have been used traditionally for various purposes, especially, in the preparation of food and as a seasoning agent. It is usually consumed fresh and the importance of onion lies in its use as a flavour enhancer in foods, due to its composition. Onion is high in dietary fibre and sugar, and about 90 percent water. Foods rich in vegetables have been known to provide numerous health benefits, as well as, prevent diseases (Slavin & Lloyd, 2012). Onions contain potassium, selenium, vitamins C, B1 and B2, the flavonoids quercetin and kaempferol, and anthocyanins (Dias, 2019; Chakraborty et al., 2022). In onion preparations, certain factors affect the content of the organo-sulphur compounds, such as, the variety (Yang, Meyers, Van der Heide, & Liu, 2004) and the processing and/or extraction conditions.

Onions are beneficial to health; this is mainly as a result of the abundance of flavonoids in them. Additionally, several studies have been conducted over the years to identify the biological effects of other onion constituents, their pharmacological and biological activities, such as antitumor, antifungal, antithrombotic, antibacterial, hypocholesterolemic and anti-inflammatory effects (Matsuura, 2001; Lanzotti, 2006). The main constituents of onion according to the report of Corzo-Martinez and Villamiel (2012) are shown in Table 2.1.

Table 2.1: General composition of onion

Component	**Content**
Energy	23.00-38.00 Kcal 100 g $^{-1}$ fresh weight
Carbohydrates	5.20-9.00 %
Protein	0.90-1.60 %
Fat	0.20 %
Ash	0.60 %
Vitamins:	**mg 100 g $^{-1}$ fresh weight**
Ascorbic acid	10.00 mg
Biotin	0.90 µg
Folic acid	16.00 µg
Nicotinic acid	0.20 mg
Pantothenic acid	0.14 µg
Riboflavin	0.05 mg
Vitamin D	0.30 mg
Elements:	**mg 100 g $^{-1}$ fresh weight**
Ca	190.00-540.00
Cu	0.05-0.64
P	200.00-430.00
K	80.00-110.00
Mg	81.00-150.00
Na	31.00-50.00
Mn	0.50-1.00
Al	0.50-1.00
Sr	0.80-7.00
Fe	1.80-2.60
Ba	0.10-1.00
B	0.60-1.00
Zn	1.50-2.80
S	50.00-51.00
Mn	0.50-1.00

Source: Corzo-Martinez and Villamiel (2012)

2.5.2 Biochemical and pharmacological properties of onion

1. Antimicrobial activity

In several societies and for centuries, onion has been used in folk medicine against bacterial, fungal, and viral infections. Its potent antimicrobial activity is the reason onion could be used as a natural preservative in foods to control the growth of microorganisms (Pszczola, 2002).

Extensive studies and chemical characterization of the sulphur compounds in onion has proven that they are the primary active antimicrobial agents (Rose, Whiteman, Moore & Zhu, 2005).

Antibacterial activity: Allicin-derived organo-sulphur compounds, such as diallyl sulphide (DAS), diallyl-disulphide (DADS), are the main active antibacterial components *in vivo* (Tsao & Yin, 2001). They have been reported to induce high inhibition against some gram-negative bacteria such as some *Escherichia coli* strains or *Salmonella enteritidis*, and gram-positive bacteria of genera *Bacillus*, *Micrococcus*, *Staphylococcus*, *Streptococcus* (Corzo-Martínez, Corzo & Villamiel, 2007; Škerget, Majhenič, Bezjak & Knez, 2009).

Certain products of quercetin oxidation in onion have been reported to exert antibacterial activity against *Helicobacter pylori* and multidrug-resistant *Staphylococcus aureus* (MRSA), in addition to organo-sulphur compounds (Ramos et al., 2006; Li et al., 2012). Although, a mild effect against *H. pylori* and a weak effect against MRSA were observed with phloroglucinol-3,4-dihydroxybenzoate, quercetin, syringaresinol, and 4-O-methylquercetin (Ramos et al., 2006). Santas, Almajano and Carbo (2010) carried out a study on the antimicrobial activity of flavonol standards and ethyl acetate subfractions of methanolic extracts of three Spanish onion varieties against *Pseudomonas aeruginosa*, *S. aureus*, *Listeria monocytogenes*, *Micrococcus luteus*, *E. coli* and *Bacillus cereus*. Their findings showed that only the ethyl acetate subfraction induced microbial inhibition.

Benkeblia (2004) reported that onion essential oil extract showed a marked antibacterial activity against some pathogens, including *S. enteritidis* and *S. aureus*. Saxena, Tripathi and Singh (2010) synthesized silver nanoparticles using onion extract and demonstrated that at a concentration of 50 µg/ml, these nanoparticles presented a completely effective antibacterial activity against *Salmonella typhimurium* and *E. coli*.

Antiviral activity: In addition to sulphur compounds, it has also been reported that the major flavonoid in onion, quercetin (3,5,7,3',4'-tetrahydroxyflavone) enhances the bioavailability of some antiviral drugs and possess antiviral activity (Wu et al., 2005). The lectins, which is

present in onion, have strong anti-HIV (human immunodeficiency virus) effect (Thirumdas, Kothakota, Pandiselvam, Bahrami, & Barba, 2021). Goren, Goldman, Trainin and Goldman (2002) also discovered a novel medicinal extract derived from onion which has broad antiviral activity. The study proposed that the extract may be used to treat or prevent a variety of viral infections in humans and animals. These viral infections include retroviral infections such as herpes (oral, genital, rectal), AIDS (acquired immune deficiency syndrome), distemper, papillomavirus, flu associated influenza viruses, parvoviruses, rhabdoviruses, Epstein Barr virus, cytomegalovirus (CMV), rhinoviruses, hepatitis virus, respiratory syncytial virus (RSV), and foot and mouth disease virus.

Chen, Chou, Cheng and Ho (2011) examined the *in vitro* antiadenoviral activity of some *Allium* plants such as onion, garlic, leeks, shallots and green onions, and found that shallots presented the highest antiviral activity for both ADV41 and ADV3, followed by garlic and onions. This suggests that other phytochemical constituents found in plants of the genus *Allium* aside quercetin and its derivatives could exert a complementary influence on the antiviral activity (Jikah & Edo, 2023), especially, given the fact that onions contain more quercetin than garlic (Gorinstein et al., 2008).

Antifungal activity: The active compounds in onion have antifungal properties. The mechanism of action of these compounds include decreasing the uptake of oxygen, inhibition of proteins, lipids and amino acids synthesis, changing cell membrane lipid profile, inhibition of fungal cell wall synthesis and cellular growth reduction (Gupta & Porter, 2001). The major components in onion extracts with antifungal activity are also DAS, DADS and diallyl trisulphide (DATS) (Chakraborty et al., 2022).

Onion extracts and its essential oil induce strong antifungal effects against many fungal species, examples include *Aspergillus niger*, *Fusarium oxysporium*, *Saccharomyces cerevisea*, *Mucor* spp, *Candida tropicalis*, *Botrytis cenerea* (Irkin & Korukluoglu, 2007; Irkin & Korukluoglu, 2009; Kocic-Tanackov, Dimic, Tepic & Vujicic, 2009: Lanzotti, Romano, Lanzuise, Bonanomi & Scala, 2012). Souza, Oliveira, de Rocha and Furlong (2010) linked the antifungal activity of onions against *Rhyzopus oryzae* with its total phenolic compounds. Three solvent systems; aqueous, ethyl acetate and methanol were used to extract the phenolic compounds of onion. The results showed that the ethyl acetate and methanol extracts inhibited *R. oryzae* development efficiently (Souza et al, 2010; Sagar, Pareek, Benkeblia & Xiao, 2022).

In addition to sulphur compounds, a wide range of peptides and proteins with antifungal activity have been isolated from many *Allium* species. Examples include Ace-AMP1 peptide from onion seeds having similar sequence with plant lipid transfer proteins (Wu, He & Ge, 2011), and allicepin, a novel peptide from onion bulbs (Wang & Ng, 2004). In a study by Wu et al. (2011) in which Ace-AMP1 was highly expressed in *E. coli* as a fusion protein, they noted that the growth of many plant fungal pathogens especially, *Alternaria solani*, *F. oxysporum* f.sp. *vasinfectum* and *Verticilium dahliae*, were inhibited by the purified protein.

Antiparasitic activity: Studies on the antiparasitic activity of onion is lean, nevertheless, available works on antiparasitic properties of onion extracts have reported positive results against different strains of *Leishmania* and *Trichomonas vaginalis* (Saleheen, Ali & Yasinzai, 2004; Taran, Rezaeian & Izaddoost, 2006).

2. Antioxidant potential

Macromolecules like the DNA (deoxyribonucleic acid), lipids and proteins are oxidized by reactive oxygen species (ROS). This plays a crucial role in aging and a variety of common degenerative diseases like cancer, inflammatory, cardiovascular, and neurodegenerative illnesses like Alzheimer's disease and other age-related degenerative conditions (Liu, Su & Guo, 2018). Numerous studies have shown that diets high in vegetables and fruits contain antioxidants like vitamins C and E, phenolic compounds (flavonoids), glutathione, and vegetable pigments in good quantities. These phytochemicals help prevent cellular damage (Dimitrios, 2006).

Red onions' anthocyanin pigments, which are concentrated in the outer layer, make up a small amount of the edible component. While kaempferol can be found in some types of onions, it is significantly less abundant than quercetin (Bora & Sharma, 2009). Accordingly, quercetin, which is present in its conjugated form as quercetin 3',4'-O-diglycopyranoside, quercetin 4'-O-glycopyranoside, and quercetin 3',7,4'-O-triglycopyranoside, is the primary flavonoid identified in onions (Sellappan & Akoh, 2002).

The onion's dry outer layers, considered as and constitutes waste has significant amounts of quercetin glycoside, quercetin, and their oxidative products, which are powerful antioxidants against oxidation of non-enzymatic lipid peroxidation and low density lipoproteins (LDL) (Gülsen, Makris & Kefalas, 2007; Bora & Sharma, 2009). The strongest antioxidative activity, that compares very well with α-tocopherol is exhibited by quercetin and its dimerized derivative (Ly et al., 2005; Park, Kim & Kim, 2007).

According to Lee et al. (2007), heating increases the physiologically active compounds of onion, as they observed that the antioxidative activities of the ethyl acetate fraction were higher in the heated (120, 130, and 140 °C) than in the raw onion, and the radical and nitrite scavenging activities increased with increase in heat treatment temperature. Woo et al. (2007) discovered similar results and concluded that 2 hours and 130 °C were the ideal heating times and temperatures. According to Roy, Takenaka and Isobe (2007), analysis of water soluble extracts of onion heated at 75 or 100 °C for 30 and 60 min, showed that both the pro-oxidant components and the overall antioxidant activity were increased.

3. Anticarcinogenic and antimutagenic activities

The anticarcinogenic or chemopreventive effects of onions and related species of *Allium* have been the subject of several epidemiologic, *in vitro*, and *in vivo* laboratory studies (Bianchini & Vainio, 2001; Galeone et al., 2006; Roldán-Marín, 2009). Onion consumption has been shown to be inversely correlated with the incidence of cancer of the stomach, indicating that onions have a preventive effect against gastric cancer (Hsing et al., 2002; Gonzalez et al., 2006; Kim & Kwon, 2009; Bang & Kim, 2010). The antibacterial qualities of onions may contribute to their chemopreventive benefits against stomach and esophageal cancers. Inhibiting bacterial development in the gastric cavity may reduce the amount of nitrate that is converted to nitrite in the stomach, the likelihood of endogenous formation of carcinogenic N-nitroso compounds, and particularly, *H. pylori* infection (Mard, Khadem Haghighian, Sebghatulahi & Ahmadi, 2014; Toh & Wilson, 2020).

Consumption of onion is consistently associated with lower risk of a good number of cancers, examples include, lung (Le Marchand, Murphy, Hankin, Wilkens & Kolonel, 2000), liver (Fukushima et al., 2001), prostate (Hsing et al., 2002), colorectal (Millen et al., 2007; Taché, Ladam & Corpet, 2007), and skin (Byun et al., 2010) cancers. These anticancer effects seem to be mediated by a number of poorly understood mechanisms. Some studies suggest the following as potential pathways by which onions exert their antimutagenic and anticarcinogenic effects; alteration of metabolism of carcinogens through induction of phase II enzymes such as UDP-glucuronosyl transferase, NAD(P)H dependent quinine reductase, and glutathione S-transferase (GST) (Tsuda et al., 2004); increasing the polarity of carcinogens and facilitating their excretion from the body (Guyonnet, Belloir, Suschetet, Siess & Le Bon, 2001; Brisdelli, Coccia, Cinque, Cifone & Bozzi, 2007); inhibition of procarcinogens bioactivating enzymes (Muto, Fujita & Yamazaki, 2001; Lautraite, Musonda, Doehmer, Edwards &

Chipman, 2002; Platt, Edenharderb, Aderholda, Muckelc & Glattc, 2010); inhibition of oxidative damage through their antioxidant action (Mutoh et al., 2000; Raso, Meli, Di Carlo, Pacilio & Di Carlo, 2001).

According to Bang and Kim (2010), onion inhibits early-stage hepatocellular carcinogenesis by suppressing oxidative stress through the modulation of the activities of glutathione peroxidase and GST; inhibiting proliferation of cells by inhibiting cell division and inducing apoptosis (Brisdelli et al., 2007). Also by, inhibiting gene transcription; and protecting against UV-induced hepatocellular carcinogenesis; and inhibiting lipoxygenase and cyclooxygenase activities (antiinflammatory effect) (Raso et al., 2001; Rose et al., 2005).

Studies have demonstrated that the anticarcinogenic activity of onions is provided, at least in part, by sulphur compounds that are both water- and lipid-soluble. Among them, DAS, DDS, dipropyl disulphide (DPDS), dipropyl sulphide (DPS), N-acetylcysteine, S-allyl cysteine (SAC), and S-methylcysteine (SMC) have been found to inhibit early and late stages of esophagus, colon, forestomach, lung, mammary gland, liver, and kidney cancers (Fukushima et al., 2001; Guyonnet et al., 2001; Bora & Sharma, 2009).

According to Viry et al. (2011), organo-sulfur compounds like the tetrasulfides found naturally in onions have the ability to inhibit the growth of both resistant and sensitive human breast carcinoma cells by specifically targeting the cell cycle's essential 25 phosphatases. Organo-selenium compounds, in addition to organo-sulphur compounds, play a significant role in the anticarcinogenic activity of onions (El-Bayoumy, Chae & Upadhyaya, 2006), achieved in part by substitution of S with Se. Compared to their comparable S-analogues, the pure Se-compounds have shown to be more effective anticancer agents. El-Bayoumy, Sinha, Cooper and Pinto (2010) stated that El-Bayoumy and colleagues in 1996 reported that diallyl selenide, for instance, is at least 300 times more effective than DAS at shrinking animal cancer tumours. The γ-glutamyl-Se-methyl selenocysteine and Se-methyl selenocysteine are the two main Se-compounds with anticancer activity in onions, with the latter having the most chemopreventive effect (Block et al., 2001). Selenomethionine, selenocysteine, and selenite/selenate are other forms of selenium that have been found in raw onions (Kotrebai, Tyson, Uden, Birringer & Block, 2000).

The presence of phenolics, especially flavonoids, in onions may also contribute to their antimutagenic and anticarcinogenic characteristics. As a function of their total phenolic and flavonoid content, including quercetin, studies have reported the various anticancer activity of

extracts from the peels and flesh of red, white, and yellow onions. In general, onion peel, which has the highest concentrations of total phenolics and flavonoids, suppressed the growth of a number of human cancer cell lines more effectively than onion meat, including cells from breast, stomach, prostate and colon cancers (Jang & Lim, 2009), as well as cells from other cancers (Jeong, Heo, Choi & Shim, 2009). Additionally, although having a larger quantity of total phenolics and flavonoids, white onion extract had lower effectiveness than yellow and red onion extracts.

Similarly, Singh et al. (2009) suggested that the high concentration of polyphenols present in the ethyl acetate fraction, including ferulic, gallic, protocatechuic acids, kaempferol and quercetin, may be the reason for the strong antimutagenic and antioxidant properties of several extracts of different polarity from red onion peel.

Specifically, quercetin and its derivatives have been shown to have anticancer characteristics in studies on a number of cancers, including breast, lung, skin, liver and prostate cancers (Le Marchand et al., 2000; Le Marchand, 2002; Vijayababu et al., 2006; Arung et al., 2011). Quercetin has also been shown to increase the bioavailability of some anticancer medications, including Tamoxifen (a non-steroidal antiestrogen) used to treat and prevent breast cancer (Wu et al., 2005; Tang et al., 2020), by decreasing metabolism and promoting intestinal absorption.

Besides quercetin, luteolin (3,4,5,7-tetrahydroxyflavone), a natural flavonoid abundant in onions, has been demonstrated to possess anti-inflammatory (Ueda, Yamazaki & Yamazaki, 2003), antiproliferative (Lin, Shi, Wang & Shen, 2008), antioxidative (Manju, Balasubramaniyan & Nalini, 2005), antiangiogenic (Bagli et al., 2004), and antimetastatic (Lee, Wu, Chen, Wang & Tseng, 2006) activities. Lutein prevents chemically-induced skin carcinogenesis in mouse models (Ueda et al., 2003). Byun et al. (2010) recently demonstrated that the activities of protein kinase C (PKC) and c-Src kinase, two protein kinases strongly linked to the development of UV-induced skin cancer, are directly suppressed by luteolin to exert its protective effect against UVB-induced skin carcinogenesis in SKH-1 hairless mice.

In addition to all of these bioactive substances, fresh chemicals are being identified and described from onion extracts. Among them, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyranone and 5-hydroxy-3-methyl-4-propylsulfanyl-5H-furan-2-one have demonstrated the ability to prevent or inhibit cancer cell growth *in vitro* by inducing apoptotic cell death through the inhibition of NF-κB (Ban et al., 2007) and by increasing the quinone reductase activity, a phase II xenobiotic metabolizing enzyme (Xiao & Parkin, 2007), respectively.

4. Cardiovascular protective effects

Cerebrovascular disease, heart attacks, hypertension, rheumatic heart disease, peripheral artery disease, heart failure and congenital heart disease, are all examples of cardiovascular disorders (CVD). Despite changes in lifestyle and advancements in CVD prevention and treatment over the past few decades, CVD continue to have a significant influence on mortality and quality of life for human populations around the world (Roldán-Marín, 2009; Wensing et al., 2009). There are numerous risk factors for cardiovascular diseases and arteriosclerosis, including high blood pressure, high cholesterol, diabetes, high triacylglcerols, LDL cholesterol, elevated blood homocysteine, increased platelet activity, which can result in the formation of arteriosclerotic plaques, and obesity.

The study conducted by Galeone et al. (2009) stated that onion has antithrombotic, hypoglycemic and hypolipidemic properties, therefore, it could be helpful in a CVD-preventive diet.

Effects on serum lipids levels: In experimental animals fed a high-fat diet, onion was shown to have exhibited the ability to lower blood triacylglcerols levels moderately and limited cholesterol biosynthesis in the liver (Ostrowska et al., 2004; Gabler et al., 2006; Roldán-Marín et al., 2010). According to Emmanuel and James (2011), there are no appreciable differences between the hypocholesterolemic and hypolipidemic effects of garlic and onion. Additionally, a study found that consumption of concentrated onion extracts had positive effects on dyslipidemia by lowering serum levels of LDL- and total cholesterol in persons with borderline hypercholesterolemia (Lee, Lee, Park & Chung, 2010).

Organo-sulfur compounds are the most active bioactive substances in onions that have hypolipidemic and hypocholesterolemic effects in both humans and experimental animals (Liu & Yeh, 2002). It has been demonstrated that the lipogenic effects of diets high in sugar, alcohol, and cholesterol can be countered by the volatile oil of onions and S-methyl cysteine sulphoxide (SMCS) (Bora & Sharma, 2009; Kumari & Augusti, 2007).

The mechanisms of action of these bioactive compounds present in onion in exerting their hypocholesterolemic and hypolipidemic activities include; inhibition of hepatic biosynthesis of lipid/cholesterol by activation of thiol enzymes (e.g., HMGCoA); reduction of nicotinamide adenine dinucleotide phosphate (NADPH) in tissue so that they may be unavailable for synthesis of cholesterol; and enhancement of turnover of cholesterol to bile acids and its excretion via the gut (Gupta & Porter, 2001).

Flavonoid quercetin and its derivatives have also demonstrated the ability to lower serum levels of LDL- and total cholesterol and increase serum levels of HDL-cholesterol (Glasser, Graefe, Struck, Veit & Gebhardt, 2002; Lee et al., 2011), in addition to organo-sulfur compounds.

Hypotensive and bradycardic effects: High blood pressure has been reported in epidemiological studies to be one of the main risk factors for coronary heart disease and stroke. When the systolic/diastolic blood pressure remains greater than 140/90 mmHg, blood pressure correlates strongly with the prevalence of cardiovascular illnesses. Many *in vivo* animal studies have demonstrated the hypotensive activity of onions. Diet containing 5 % dried onion lowered blood pressure in L-NAME (L-N^G-nitro arginine methyl ester)-induced hypertensive rats, and stroke-prone spontaneously hypertensive rats (SHRSP) (Sakai, Murakami, & Yamamoto, 2003).

Studies have shown that the active components in onions that lower blood pressure do so most likely through raising levels of nitric oxide (NO) and the activity of nitric oxide synthetase (NOS). Studies using human umbilical vein endothelium cell culture (Jiemei, Jianjun, Jie, Benhong & Hao, 2011) and SHRSP (Sakai et al., 2003) have both shown this to be the case. In addition, it has been demonstrated that the flavonoids, phenolics, and 3-mercapto-2-methylpentan-1-ol (3-MP) of onions scavenged peroxynitrite radical *in vitro*, inhibiting the peroxynitrite-induced nitration of protein tyrosine residues, which is thought to be one of the primary causes of many human diseases, including CVD (Ho et al., 2010; Rose et al., 2003). According to a study by Sanchez et al. (2007), the antioxidants quercetin and isorhamnetin (quercetin's methylated metabolite), which are found in onions, can lower blood pressure and prevent angiotensin II-induced endothelial dysfunction. These are achieved through the inhibition of the over-expression of p47 (phox), a regulatory subunit of membrane reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, and the subsequent increase in superoxide production, which results in the highest NO bioavailability (Sanchez et al., 2007); and through calcium influx inhibition (Naseri, Arabian, Badavi & Ahangarpour, 2008).

A capsule formulation of onion-olive oil maceration was tested in a randomized, double-blind, placebo-controlled, crossover research by Mayer et al. (2001) to see how arterial blood pressure can be affected by it. They discovered that it caused an arterial blood pressure reduction. Another study examined the effectiveness of quercetin supplementation in lowering blood pressure in hypertensive patients and found that quercetin at 730 mg per day could lower stage 1 hypertensive patients' mean arterial pressures, systolic blood pressure, and diastolic blood

pressure by 5, 7, and 5 mmHg, respectively (Edwards et al., 2007). Similarly, another randomised, placebo-controlled, double-blinded, cross-over study by Egert et al. (2009) demonstrated that quercetin decreased plasma oxidized LDL concentrations and systolic blood pressure in obesed patients with high-CVD risk profile.

Anti-hyperglycemic/Anti-diabetic activity: Studies on diabetic animal models and humans have revealed the usefulness of onions and their derivatives as hypoglycemic agents (El-Demerdash, Yousef & Abou El-Naga, 2005; Srinivasan, 2005). According to research carried out by Bang, Kim and Cho (2009), the ability of onions to reduce renal oxidative stress, and lower serum levels of lipid/cholesterol, blood glucose in streptozotocin-induced diabetic rats may be the mechanism through which onions have a good ameliorative effect on diabetic nephropathy. Masood et al. (2021) found that onion supplemented diet improved body weight and significantly reduced blood glucose levels in diabetic rats. In experimental animals with type 2 diabetes mellitus, Lee et al. (2008) demonstrated that onion peel was helpful in regulating hyperglycemia in part via reducing α-glucosidase activity.

According to studies by Urios, Grigorova-Borsos and Sternberg (2007), long-term quercetin absorption may be helpful in preventing the advanced glycation of collagen, which contributes to development of cardiovascular problems in diabetic patients. Another study undertaken by Jung, Lim, Moon, Kim and Kwon (2011) using high-fat diet/streptozocin-induced diabetic rats demonstrated that the extract of onion peel, which is high in quercetin, may improve glucose response and insulin resistance that is associated with type 2 diabetes. This is even in a more effective manner than pure quercetin equivalent, the mechanisms included but not limited to, increasing glucose uptake at peripheral tissues, suppressing oxidative stress, and reducing metabolic dysregulation of free fatty acids. These results support the reduction of insulin insensitivity in type 2 diabetes mellitus using onion peel.

Additionally, studies have suggested that the presence of compounds such as, quercertin, polyphenols, allyl propyl disulphide, s-methylcysteine sulfoxide, in onion facilitate glucose uptake in peripheral tissues (Sabiu et al., 2019).

5. Anti-platelet/Anti-thrombotic effect

Blood platelets' primary role is to preserve the haemostatic integrity of arteries and to stop bleeding after injury through vasoconstriction, clot formation, and blood coagulation (Ali et al., 2000). A blood clot (thrombus) can obstruct blood flow through a vein or artery, and even detach from the wall of blood vessel to become a life-threatening embolus when it lodges in

the lungs or other essential organs. It can occur when there is an imbalance in the blood coagulation system.

Acute coronary syndrome occurs due to blood clots in the coronary arteries, while stroke in persons with atrial fibrillation is most commonly brought on by blood clots that form in the heart. As a result, thrombosis problems have a major impact on CVD (Alkarithi, Duval, Shi, Macrae, & Ariens, 2021).

It is known that raw onions have antiplatelet properties. However, as onions are typically fried before eating, their anti-aggregatory properties are presumably lost. According to several trials, even at high dosage levels, boiling onions had no anti-thrombotic effect. This is likely because the beneficial components degraded with time. These indicate that onions should be consumed raw or very lightly cooked in order to reap the greatest health advantages (Cavagnaro, Sance, & Galmarini, 2007).

Furthermore, it has been demonstrated that several non-sulfur chemicals, such β-chlorogenin and quercetin, can prevent platelet aggregation (Rahman, K. Allison & Lowe, 2006). Quercetin exhibits its cardiovascular health benefits by inhibiting endothelial cell injury damage associated with development of atherosclerosis (Zhang & Dou, 2019).

Two phenylpropenoic acid amides; alfrutamide (N-feruloyltyramine) and typheramide (N-caffeoyltyramine), derived from *Allium fistulosum* (green onion), have been reported to have anti-inflammatory properties. The cyclooxygenases (COX) 1 and 2 are primarily responsible for catalyzing the conversion of arachidonic acid into a number of prostaglandins and thromboxanes (such as thromboxane A2 and thromboxane B2), which in turn promotes platelet aggregation. These commpounds have been proven to dramatically inhibit these enzymes (Park, 2011).

6. Other beneficial effects

Kaiser et al. (2009) assessed the antiallergic properties of the herbal fraction (ALC-02) obtained from the onion bulb in rats. The authors attributed ALC-02's antiallergic profile to its possible antihistaminic, anti-inflammatory, and antioxidant capabilities after testing the efficacy against several factors that cause Type I allergic reactions. Onion is said to stimulate the digestive system, speeding digestion and shortening the time food spends traveling through the digestive tract (Platel & Srinivasan, 2001). Naseri et al. (2008) looked at the spasmolytic effects of onion peel powder on the contractility of the rat ileum, among other things. Without

requiring the activation of β-adrenoceptors, nitric oxide generation, potassium channels, or opioid receptors, onion peel extracts obtained in 70 % alcohol reduced ileum contractions. The authors hypothesized that the quercetin in onion peel extracts would cause a spasmolytic effect by activating calcium channels.

Due to the high soluble fibre content of onions, particularly fructooligosaccharides (FOS) and inulin (Cardelle-Cobas, Costo, Corzo & Villamiel, 2009), onions encourage the growth of certain microbes, such as bifidobacteria and lactobacilli, in the colon which generally benefits human health (Ernst & Feldheim, 2000). This suggests that onion has prebiotic activity. In their study of the effects of onion extract and an onion by-product made from pasteurized paste on the gut of healthy rats, Roldan-Marn et al. (2009) found that these products had a prebiotic effect as shown by changes in pH, butyrate production, and gut microbiota enzyme activity.

Khaki et al. (2009) also observed that freshly prepared onion juice significantly affects the sperm count, motility and percentage of viability in Wistar rats. Thus, 4 g/kg of fresh onion juice boosts sperm health parameters. The consumption of onions has also been shown to be negatively correlated with BPH. This was noticed by Galeone, Tavani, Pelucchi, Negri and La Vecchia (2009) in a multicenter case-control study with 1369 patients with BPH and 1451 controls.

2.6 *Annona muricata*

2.6.1 Description of *Annona muricata*

The scientific classification of *Annona muricata* is as follows; Kingdom: Plantae; Division: Tracheophyta; Class: Magnoliopsida; Order: Magnoliales; Family: Annonaceae; Genus: *Annona*; Species: *Annona muricata* L. (United States Department of Agriculture, n.d).

Plate 2.2: *Annona muricata* fruit (https://www.istockphoto.com/photo/annona-muricata-soursop-fruit-isolated-on-white-gm1210614847-350788933)

Annona muricata is known as soursop in English, graviola in Portuguese, guanábana in Latin American Spanish and other indigenous names. *Annona muricata* is the most widely grown species of the genus *Annona* comprising of over 70 species. Its synonyms are *Annona muricata* var. Borinquensis Morales, *Annona bonplandiana* Kunth, *Annona cearensis* Barb. Rodr., *Annona macrocarpa* Wercklé; and *Guanabanus muricatus* M. Gómez (Pinto et al., 2005). In Nigeria, it is called "Ebo" or "Apekan" in Yoruba, "Fasadarur" or "Tuwon biri" in Hausa and "Sawansop" in Igbo (), although it is best known as "Sawasop" (Badrie & Shauss, 2010).

The soursop tree grows to about 5 to 10 m in height and 15 to 83 cm in diameter with low branches (Benavides, 2003). It is found in the tropical regions of Central and South America, Southeast Asia and Western Africa at altitudes below 1200 m above sea level, temperatures of 25 to 28 °C, annual rainfall of more than 1500 mm and relative humidity of 60 to 80 %. It blooms and fruits through most of the year, but depending on altitude there are more distinct seasons. The fruit (Plate 2.2) is an edible, dark green and ovoid berry; with white and creamy flesh that has a characteristic flavour and aroma (Pinto et al., 2005). In some countries, the average weight of the fruit is 4 kg (Pinto et al., 2005), while varying between 0.4 and 1.0 kg in México (Evangelista-Lozano, Cruz-Castillo, Pérez-González, Mercado-Silva & Dávila, 2003), Venezuela (Ojeda et al., 2007) and Nicaragua (Benavides, 2003). When fresh, each fruit contain about 55 to 170 black seeds that turn light brown when dry.

This fruit has become popular as a result of bioactive substances with potentials as nutraceuticals. If ingested in moderation, the pulp is a great source of fibre and includes various substances that promote health. For instance, the polyphenols in soursop plants are organic substances with antioxidant potentials that protect cells from the damaging effects of free radicals (Gonzalez et al., 2017). This plant contains more than 200 distinct bioactive substances, the bulk of which are alkaloids, phenols, and acetogenins (Gyesi et al., 2019).

Phytochemicals from soursop have a long history of usage in herbal medicine. They can be used to treat diseases like cancer, bacterial or parasitic infections, fever, high blood sugar, high blood pressure, inflammation, and anxiety. Various scientific interventions have shown that soursop extract posseses antibacterial, antiprotozoan, anti-inflammatory, and anticancer effects (Gyesi et al., 2019).

2.6.2 Traditional medicinal uses

The bark, leaves, seeds, and fruits *Annona muricata* have been used for numerous purposes in traditional medicine (Badrie & Schauss, 2009). The most common form of use being the

decoction of leaves, bark, root, or seeds, in various ways. The leaves are used in baths to treat skin diseases in Indonesia, and the Caribbean islands (Boulogne, Germosén-Robineau, Ozier-Lafontaine, Fleury & Loranger, 2011). The fruit is used as food, while the juice is used as galactogogue to treat diarrhoea, liver and heart diseases, and against intestinal parasites in South America (Badrie & Schauss, 2009). Recently, the leaves of *A. muricata* are being used in the treatment of diabetes (Badrie & Schauss, 2009) and cancer (Alonso-Castro et al., 2011).

The roots, unripe fruit, leaves and seeds of *Annona muricata* are also used as topical insect repellents, bioinsecticides and biopesticides. Brechelt (2004) recommended the use of this plant in pest control and the use of the aqueous extract of soursop to control aphids, thrips, and lepidopteran larvae.

2.6.3 Nutritional composition

The soursop fruit's weight breakdown is as follows: 4 % core, 8 % seeds, 20 % skin, and 67 % edible pulp. Their pulp is abundant in B1, B2, and C vitamins, nonreducing sugar, water, protein, and carbohydrates (Badrie & Schauss, 2010). Refractive indices of 1.335 for the seeds and 1.356 for the pulp, soluble solid contents of 151 Brix for the pulp and 1.51 Brix for the seeds, and pH 4.56 for the pulp and 8.34 for the seeds, were among the fruit's physicochemical properties. Sugars make up about 70 % of the total solids in the soursop pulp, coming second in abundance only to water. A high percentage of the total sugar content, 93.6 %, is made up of glucose and fructose (Afzaal et al., 2022). The soursop pulp's fructose, D-glucose, and sucrose concentrations, measured using gas-liquid chromatography, were found to be 1.80, 2.27, and 6.57 %, respectively, for 10.48 % total sugar content. The soursop fruit has calcium chloride, salt, potassium, citrate, pectin, and 12 % sugar (mainly glucose with some fructose) (George, Kumar, Suresh & Kumar, 2015). According to reports, the pulp from soursop has between 0.78 and 0.95% fibre. Pectin, which makes up 0.91 % of the fresh weight of ripe fruit on an alcohol-insoluble basis, was the predominant substance. On dry weight basis, the fraction declines from climacteric to climacteric phases from 12.0 to 4.0 %. The pulp weighs 0.055 gN/100 g when moist; out of which 91 % was contributed by the acid and free amino acids. Paper chromatography was used to identify 11 free amino acids, and four more unidentified ninhydrin-positive compounds. The most abundant free amino acids were proline and c-aminobutyric acid. Glycine, serine, aspartic acid, lysine, glutamic acid, citrulline, alanine, arginine and cysteine were among the other amino acids detected (Yahaya, 2019).

In Ibadan, southwest Nigeria, a study was conducted on the preharvest degradation of soursop and its impact on nutrient composition. The fresh non-infected soursop fruits that were gathered were analyzed for nutrients, and in the five test sites, they contain 14.88 to 14.91 % carbohydrates, 1.20 to 1.24 % crude protein, 0.89 to 0.90 % ash, 78.49 to 78.68 % moisture content,19.15 to 19.35 % dry matter, 1.39 to 1.41 % potassium and 0.63 to 0.65 % salt (Amusa, Ashaye, Oladapo & Kafaru, 2003). Comparable values have been established. In the freshly harvested fruits, a 39 % drop in the amount of carbohydrates has been noted. This was most likely caused by the infections' deteriorating actions, which reduced the fruit's quality. The decrease in crude protein and dry matter for the contaminated fruits was roughly 20 and 11 %, respectively. However, compared to uninfected fruits, infected fruits had increased ash and moisture contents (Badrie & Schauss, 2010). The fruit is a good source of fibre, vitamins, and minerals (Olagunju & Sandewa, 2018).

Around 66 % of the weight of the fruit is made up of the edible pulp, which has a delicious aroma and flavour. It is a good source of minerals and vitamins. Some of their chemical makeup are divided into different classes (Table 2.2) (Yathzamiry et al., 2021). Phenolic acids and flavonoids are two prominent examples. An analysis of the phytochemical composition of methanol extract found alkaloids and flavonoids. Both hexane and ethyl acetate extract contained tannins and phenolic components (Yahaya, 2019). To enhance the nutritional and functional qualities of yoghurt and frozen desserts, Virgen-Ceceña et al. (2019) incorporated soursop pulp. The study found that the strong antioxidant activity of these value-added food products is provided by dietary fibre, polyphenols, and ascorbic acid.

Afzaal et al. (2022) suggested that the soursop seeds might be harnessed for human and animal nutrition because they are rich in protein and oil, although it also contains intoxicants such as tannins, cyanide, and phytate. The seeds contain 21 % protein and 22 % pale yellow oil. The oil has a bland flavour and the following chemical compositions: 0.93 for acidity, 227.48 for saponification, 111.07 for iodine, and 66.77 for acetyl, 28.07 % saturated fatty acids and 71.93 % unsaturated fatty acids. The seeds produce a yellowish-brown, 70 % unsaturated oil, with traces of myristic acid, that also made up of 16 % palmitic, 12 to 33 % linoleic, 5 % stearic and 41 to 58 % oleic acids. This oil may be edible if some potentially dangerous components can be removed. The pulp has a higher level of calcium and potassium than the peel or seeds, whereas the seed has a higher content of zinc and magnesium than the pulp. The seeds contain 17.0 mg of calcium per 100 g, 0.2 % water-soluble ash, and 0.79 % titratable acidity (Onimawo,

2002; Solis-Fuentes, Hernandez-Medel & Duran-de-Baz´ua, 2020). The nutritional composition of soursop fruit according to Afzaal et al. (2022) is shown in Table 2.2:

Table 2.2: Nutritional composition of soursop fruit

Nutritional components	**Amounts**
Antioxidant components:	**(mg/100 g)**
Total phenolic compounds	449.47
Total flavonoid compounds	1.97
Total anthocyanin content	0.15
Organic acids	**(mg/100 g)**
Acetic acid	1.66
Lactic acid	15.56
Oxalic acid	0.02
Citric acid	1.43
Sugar content:	**(mg/100 g)**
Glucose	0.41
Fructose	0.62
Sucrose	1.86
Proximate composition:	**(%)**
Moisture	67.45
Protein	3.59
Fat	0.80
Crude fibre	2.36
Ash	1.98
Soluble fibre	5.40
pH	3.87
Nutritional profile:	**(mg/100 g)**
Thiamine	0.11
Niacin	1.28
Riboflavin	0.05
Ascorbic acid	29.6
Methionine	7.00
Tryptophan	11.00
Lysine	60.00 g

Source: Afzaal et al. (2022)

2.6.4 Therapeutic potentials of soursop

An examination of phytoconstituents and bioactive components of *Annona muricata* L. found that they have therapeutic qualities. The soursop leaf has been found to effectively inhibit the growth of cancerous cells by causing apoptosis, enhancing immunological response, lowering blood glucose levels, alleviating depressive symptoms, promoting digestion, and dilation of blood vessels (Badrie & Schauss, 2010).

1. Antioxidant potential

Plant products that are rich in a variety of functionally active compounds with antioxidant potential, may be able to lower the amount of free radicals created in excess through metabolic processes and various external stressors. Consuming dietary antioxidants that help the body fight free radicals is necessary because high amounts of free radicals produce oxidative stress, which can damage tissue, DNA, proteins, and lipids (Reshi, Kaul, Bhat & Kaur, 2012). The presence of flavonoids and tannins in soursop extracts may be essential for its ability to scavenge free radicals (Meza-Gutierrez et al., 2022). Alkaloids, carbohydrates, flavonoids, tannins, phenols, coumarins, saponins and terpenoids, for example, are therapeutically useful compounds found in soursop leaves (Nguyen et al., 2020).

According to a study, the ethanol extract of the soursop plant contains acetogenins, a class of phytochemicals with modest antioxidant activity, as well as alkaloids, anthraquinones, saponins, flavonoids, lactones, terpenoids, phenols, phytosterols and coumarins (Daud, Ya'akob & Rosdi, 2016). These phytochemicals have a strong inhibitory effect on some enzymes, especially in the membranes of tumor cells (Afzaal et al., 2022).

The ability of antioxidants to lower reactive oxygen species is associated with their ability to inhibit chain initiation and deactivate excited molecules, such as singlet molecular oxygen and peroxyl radicals. The petroleum ether fractions from the leaves and stem bark have greater abilities to scavenge hydroxyl-free radicals (Agu & Okolie, 2017). The fruit pulp and leaf of the soursop have a higher antioxidant capacity than the other plant parts, since phytochemicals and other phytoconstituents such as phenols, alkaloids, flavonoids and vital lipids are in large quantities in these parts (Agu & Okolie, 2017; Afzaal et al., 2022). These results led to the conclusion that there may be a link between the soursop's ethnomedicinal qualities and its antioxidant potentials (Agu & Okolie, 2017).

2. Antidiabetic effect

A shift in blood glucose levels brought about by abnormalities with insulin sensitivity, insulin secretion, or both is known as hyperglycemia. The reduction of hyperglycemia is a crucial therapeutic approach in the management of diabetes. However, blocking the major enzymes amylase and glucosidase that are in charge of breaking down starch and assimilating glucose would offer a nutraceutical option for treating the disease. Blood glucose levels are significantly influenced by the activity of the enzymes amylase and glucosidase; inhibiting these enzymes may significantly lower blood glucose levels after eating.

Standard techniques were used to determine the *in vitro* inhibitory characteristics of methanol, ethyl acetate, and dichloromethane extracts from various sections of the plant on α-amylase and α-glucosidase activities. The manner and mechanism of interactions between extracts (isolated acetogenin) and enzymes were identified. According to this study, the pulp of the soursop fruit can inhibit the activity of the enzymes α-amylase and α-glucosidase and reduce blood fat deposition (Agu et al., 2019).

According to another study, the activity of the enzymes α-amylase and α-glucosidase significantly affected blood sugar levels, and blocking these enzymes can significantly reduce blood sugar levels after eating a meal (Gong et al., 2020). Amylase and glucosidase would be inhibited by the aqueous extract of seed coat of the soursop fruit as it contains the highest total phenol and flavonoid content (Akomolafe & Ajayi, 2015). The α-amylase and α-glucosidase activities were suppressed by the plant because of the phenolic content (Afzaal et al., 2022). Additionally, it was shown that soursop extracts significantly inhibited α-glucosidase more than α-amylase, which is crucial for medicinal purposes. The soursop plant's phenolic-rich extract decreased α-glucosidase activity more than α-amylase activity (Adefegha, Oyeleye & Oboh, 2015). Additional studies suggest that soursop seed oil may also help to lower type 1 diabetes (Pinto et al., 2018).

The soursop leaf aqueous extract is used to increase resistance to glucose load and decrease gastrointestinal glucose uptake (Guevara-Vasquez, Campos-Florian & Davila-Castillo, 2021). In hyperglycemic individuals, *A. muricata* extracts may lower blood sugar levels while restoring antioxidant enzymes such as catalase, and superoxide dismutase (SOD), and nitric oxide. Additionally, soursop has been demonstrated to have a protective effect against liver lipid peroxidation when consumed (Alwan, Lim, Samad, Widyawati & Yusoff, 2020).

3. Effect on cholesterol

Familial hypercholesterolemia (FH), a hereditary condition that can advance to early coronary heart disease (CHD), is characterized by elevated LDL-cholesterol (LDL-C) levels (Di Taranto et al., 2020). Fruit pulps with fragrant compounds, such *Annona muricata*, may have favourable metabolic effects, especially on lipid status. Agu and Okolie (2020) observed decrease in plasma levels of low-density lipoprotein (LDL), triglycerides (TGs), and very low-density lipoprotein (VLDL) cholesterol as a result of the effects of soursop bioactive components on basal lipid profile and plasma fatty acid synthase (FAS) activity. Flavonoids, tannins, and alkaloid compounds found in soursop leaf extract lower blood cholesterol. People take three to five doses of soursop leaf extracts daily, which is prepared by boiling soursop leaves in two to three glasses of water until a half or one glass remains to lower cholesterol levels (Afzaal et al., 2022). Plasma total cholesterol can be decreased by 96 % with ethanol extract of soursop leaves (Ramatillah, Astiani & Cecylia, 2017).

4. Effect on blood pressure

The force of flowing blood exerted on the walls of blood arteries is known as blood pressure. The term "hypertension" refers to high blood pressure. Congestive heart failure, stroke, peripheral artery disorders, and renal diseases are all made more likely by high blood pressure (American Heart Association, 2017).

Soursop fruit contains phenolic compounds and alkaloids that help reduce blood pressure and decrease the generation of uric acid in people with hyperuricemia (Nayak & Hedge, 2021). Several studies have demonstrated that utilizing soursop fruit supplements can lower blood pressure and serum uric acid levels following a 12-week therapy period. Patients with hyperuricemia were given soursop leaf n-butanol extract at doses of 250 and 500 mg/kg body weight, which reduced further uric acid generation and controlled blood pressure (Alatas et al., 2020).

5. Antihemorrhagic effect

Humans frequently get hemorrhoids, which typically develop in the rectum. External hemorrhoids develop under the skin near the anus. Hemorrhoids are more frequent in adults between the ages of 45 and 65, though the actual incidence is difficult to estimate because so many sufferers are unwilling to admit (Aziz, Huin, Badrul-Hisham, Tang & Yaacob, 2018; Swartz & Wright, 2020). Hemorrhoids, inflammatory bowel disease, and autoimmune

disorders can all be helped by consuming plants high in phenolic components such as flavonoids, catechins, tannins, phenolic acid, and stilbenoids (Shahidi & Ambigaipalan, 2015).

Annona muricata contains phenolic components including quercetin and gallic acid, as well as phytochemicals such acetogenins, alkaloids, and megastigmanes that are known to have anti-inflammatory and antioxidant activities. Because the extract from soursop leaves contains anti-inflammatory properties, it can be utilized to treat hemorrhage (Ayun, Kusmardi, Nurhuda & Elya, 2020). A natural mixture of bioactive substances with the ability to promote biogenesis - the production of new blood vessels from pre-existing ones - can be found in the soursop leaf aqueous extract (Cardenas et al., 2021).

6. Anticarcinogenic effect

The annonaceous acetogenins in soursop leaf demonstrated potent inhibitory actions against human malignancies including those of the lungs, breast, small intestine, liver, and kidneys (Jacobo-Herrera et al., 2019). Herbal medicines can be a helpful or complimentary adjunct to conventional cancer therapies. (Berumen-Varela et al., 2020).

The fruit's anonaceous acetogenins have demonstrated notable anticancer effects (Afzaal et al., 2022). *Annona muricata* leaves contain 34 acetogenins that have been isolated. The acetogenins have been found to be more harmful to tumour cells than to healthy cells.

Studies on the effects of these compounds on human health indicate that natural polyphenols like flavonols, flavones, isoflavones, anthocyanins, and catechins, may be able to prevent cancer (Indrawati et al., 2017). Daddiouaissa et al. (2019) examined the cytokinetic behaviour of human breast cancer cells (MCF-7) using an ionic liquid extract of graviola (soursop) fruit (IL-GFE). The mechanism of action of IL-GFE on MCF-7 cancer cells as it affects cell cycle distribution and apoptosis was revealed. They came to the conclusion that soursop ionic liquid extract can slow the proliferation of malignant cells.

7. Pro-apoptotic properties

Apoptosis is programmed cell death triggered by internal mechanisms. Both the intrinsic and extrinsic pathways of liver disease progression, as well as mitochondrial diseases, are slowed down by apoptosis (Tejasari, Sastramihardja, Abdurachman & Prasetyo, 2018; Hadisaputri, et al., 2021). It is an essential physiological mechanism required for tissue homeostasis and healthy cell maintenance (Hadisaputri et al., 2021). Addressing apoptosis delay is beneficial

for many types of cancer, since it is the hallmark of cancer and is independent of the disease's cause or kind (Pfeffer & Singh, 2018).

Annona muricata has been proposed as a potential dietary addition for the development of anti-cancer drugs (Ilango et at., 2022). It has been suggested that *Annona muricata* could be used as a natural source for the development of medications that cause cancer cells to undergo apoptosis. It has been demonstrated that the flavonoids in this fruit are more effective in inducing apoptosis than camptothecin, a well-known anticancer drug (Afzaal et al., 2022). Nuclear transcription factors, genes involved in the cell cycle, and genes related to apoptosis have all shown altered amounts of mRNA in response to flavone activity. Additionally, flavones have been shown to specifically cause apoptosis and decrease proliferation in changed colonocytes (Indrawati et al., 2017).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Equipment/Apparatus

All the equipment and apparatus used in this study were of good standard and in good working condition. They include; centrifuge (MedGroup MSLZL09), waterbath (Hilmedics H-420), spectrophotometer (Turner® 390), test tubes (Pyrex) and test tube racks, electronic weighing balance (Sunrise SUN-224CL-220Gm), micropipettes (West tune), Pasteur pipettes, Measuring cylinders (Pyrex), Erlenmeyer flasks (Pyrex), rotary microtome (Leica RM2235), blunt nose forceps, microscopic slides and coverslips, binocular microscope (Leica DM 1000), microplate reader (Infitek MPR-H200BC), microplate shaker (Infitek SHK-M2000), microcentrifuge tubes (Pyrex), micro tissue grinders (Pyrex), sonicator (Qsonica Q55), squirt bottles, blender (Binatone BLG-595 MK2), dissecting sets, syringes and needles, capillary tubes, specimen bottles, glass funnels (Pyrex), gavage, colony counter (tt 201), autoclave (MSLPS18), incubator (DN-9022A), swabsticks, petri dishes (Pyrex).

3.1.2 Chemicals/Reagents

Analytical grade chemicals and reagents were used in this study, while the drugs used were of pharmaceutical grade. They are; ethanol, potable water, distilled water, Folin-Ciocalteau reagent, methanol, vanillin, DPPH, butylated hydroxytoluene (BHT), ABTS solution, ammonium persulphate, monopotassium phosphate, dipotassium phosphate, hydrogen peroxide solution, dimethyl sulphoxide (DMSO), ethylenediamine tetraacetic acid (EDTA), sodium nitroprusside, nitroblue tetrazolium (NBT), riboflavin, deoxyribose, ferric chloride, ascorbate, gallic acid, thiobarbituric acid (TBA), sodium phosphate dibasic heptahydrate, sodium phosphate (monobasic monohydrate), sodium hydroxide, sodium chloride, disodium phosphate, potassium chloride, Griess reagent, sodium phosphate buffer, potassium ferrocyanide, trichloroacetic acid, sulphuric acid, sodium phosphate, ammonium molybdate, testosterone (CAS 57-85-2), oestradiol (CAS 979-32-8), olive oil (CAS 8001-25-0), finasteride (CAS 98319-26-7), potassium chloride, L-methionine, triton X-100, hydroxylamaine, sulphanilamide, N(1-naphthyl) ethylenediamine (NED), sodium azide, GSH, acetic acid, sodium dodecyl sulphate, Ellman's reagent, 1-chloro-2, 4-dinitrobenzene (CDNB), potassium dichromate, protein test kit (Biosystems, U.S.A), 2,4-dinitrophenylhydrazine (DNPH),

guanidine solution, ethyl acetate, isopropyl alcohol, normal saline, formaldehyde, xylene, paraffin wax, haematoxylin, eosin, DPX mountant, NADPH **(**CAS 53-59-8), PSA test kit (Accubind® immunoenzymometric assay type 3), testosterone test kit (Accubind® competitive enzyme immunoassay type 7), prolactin test kit **(**Accubind® immunoenzymometric sequential assay type 4), dihydrotestosterone test kit (Enzyme-linked immunosorbent assay (ELISA), Diagnostic Automation, Inc.: Cortez Diagnostics), interlukin-8 test kit (ab214030 IL-8 SimpleStep ELISA® kit), tumor necrosis factor-α (ab181421 TNF alpha SimpleStep ELISA® kit), TNF-β/Lymphotoxin-α (ab229202 TNF beta SimpleStep ELISA® kit), 5-α reductase test kit (MBS2021309 ELISA kit for steroid 5-α reductase 2), lysis buffer (CAS 151-21-3), sucrose, tris(hydroxymethyl)aminometjane (CAS 77-86-1), ABCAM® kit (for immunohistochemical staining for formalin-fixed paraffin-embedded-FFPE tissue sections), trisodium citrate (dihydrate), deionized water, tween-20 sodium carbonate.

3.1.3 Culture media

The culture media used were; nutrient agar (NA), MacConkey agar (MCA), *Salmonella-Shigella* agar (SSA), triple sugar iron agar (TSI), and potato dextrose agar (PDA).

3.1.4 Plant materials

Fresh bulbs of common onion (red onion) and ripe fruits of soursop were used for this study. The plant samples (onion bulbs and soursop fruits) were purchased from Relief market, Owerri, Imo state, Nigeria. The plant samples were identified by Dr. C. M. Duru, a taxonomist in the Department of Biology, Federal University of Technology, Owerri, Imo state, with authentication numbers; FHI 110177 (*Annona muricata* L.) and FHI 107561 (*Allium cepa* L.). They were washed under running tap water. The soursop fruits were cut open to collect the pulp with the seeds removed, while the onion bulbs were cut into smaller pieces. The traditional method of making fruit juice was used to make onion bulbs and soursop pulp juice. This was done by blending the onion bulbs and soursop pulp to fine paste separately. After which, the samples were filtered with muslin cloth, and the filtrates collected for use in the study.

3.1.5 Experimental animals

Healthy thirty-six Swiss albino mice weighing 15 to 20 g were used for the acute toxicity study. While, healthy fifty-four (54) adult male albino rats of Wistar strain weighing 120 to 150 g were used for the study. The animals were purchased from the Animal House of a research

laboratory, Dave Research House, Owerri, Imo State, Nigeria. Ethical approval was obtained for the use of experimental animals in the research (see Appendix A).

3.1.6 Other materials

Other materials used in the study include; muslin cloth, Whatman no. 1 filter paper, thick gloves (for handling experimental animals, examination gloves, ice packs, metal cages, feed (Vital feed finisher pellets, Ibadan), feeders, kitchen knife, packaging bottles and caps, packaging labels, scissors, cotton wool, blotting paper, masking tape, marker pens.

3.2 Methods

3.2.1 Acute toxicity (LD_{50}) studies

The index of acute toxicity is the LD_{50}. Lorke (1983) method was used with slight modification. This method is divided into two phases.

Phase I

For each juice sample, three groups containing three mice each were used. The juice samples were administered at concentrations of 10 mg/kg body weight (b.w) to group I mice, 100 mg/kg b.w to group II mice, and 1000 mg/kg b.w to group III mice (that formed the LD_{50} phase one groups). Administration of juices was done orally. The animals were monitored for 24 hours for abnormal reaction or death.

Phase II

This was also done for both juice samples. In this phase, three groups of mice were used with the both the onion and soursop juices in the concentration of 1600 mg/kg b.w., 2900 mg/kg b.w., and 5000 mg/kg b.w. to groups 1, group 2 and group 3 mice. They were monitored for 24 hours and the observations were recorded. Median lethal dose (LD_{50}) of the juice samples was estimated by calculating the geometric mean of the maximum dose with 0 % mortality and the minimum dose with mortality.

$$LD_{50} = \sqrt{\begin{matrix}\text{maximium dose with 0 \% mortality} \times \\ \text{minimium dose with 100 \% mortality}\end{matrix}} \quad \text{(Equation 3.1)}$$

3.2.2 *In vitro* antioxidant assay

1. Ferric reducing antioxidant property

The reducing property of the extracts was determined as described by Pulido, Bravo and Saura-Calixto (2000).

Principle: The principle of the assay is the quantification of ferric degradation product, by its condensation with the sample. Antioxidants reduce the ferricyanide to ferrocyanide, which binds the free Fe^{3+} in the solution and forms Prussian blue which can be measured spectrophotometrically at 700 nm

Procedure: A volume of 0.25 ml each of the samples was mixed with 0.25 ml of 200 mM sodium phosphate buffer pH 6.6 and 0.25 ml of 1 % potassium ferrocyanide. The mixture was incubated at 50 ^{o}C for 20 minutes, thereafter 0.25 ml of 10 % trichloroacetic acid was added and centrifuged at 2000 rpm for 10 min. Then, 1 ml of the supernatant was mixed with 1 ml of distilled water and 0.2 ml of 20 mM ferric chloride and the absorbance was measured at 700 nm. Gallic acid concentrations (2 – 12 µg/ml) was used to plot the standard curve. The results were expressed as µg/ml.

2. Measurement of nitric oxide scavenging activity

The extent of inhibition of nitric oxide radical generation *in vitro* was estimated using the method reported by Green et al. (1982).

Principle: Sodium nitroprusside in aqueous solution, at physiological pH, spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that are estimated spectrophotometrically at 546 nm.

Procedure: The reaction was initiated by adding 2.0 ml of sodium nitroprusside (100 mM), 0.5 ml of phosphate buffered saline (PBS, pH 7.4), 0.5 ml of samples and incubated at 25^{o}C for 30 minutes. Exactly 0.5 ml Griess reagent (1 % sulphanilamide, 2 % H_3PO_4 and 0.1 % naphthylethylene diamine dihydrochloride) was added and incubated for another 30 minutes. Control tubes were prepared without the samples. Standard gallic acid solution at concentrations of 2 to 12 µg/ml was used to generate a standard curve. The absorbance was read at 546 nm against the reagent blank, in a spectrophotometer. The nitric oxide scavenging potential of the samples were determined from the standard curve and expressed as µg/ml.

3. Estimation of total phenol content

The amount of total phenols in the samples was estimated by the method outlined by Malik and Singh (1980).

Principle: Phenols react with phosphomolybdic acid in Folin-Ciocalteau reagent to yield a blue-coloured complex in alkaline medium, which can be measured spectrophotometrically at 650 nm.

Procedure: The sample (0.5 ml) was thoroughly mixed in 10 times volume of 80 % ethanol, and centrifuged at 10,000 rpm for 20 minutes. The supernatant was preserved. Then, the sample was re-extracted with 2.5ml 80 % ethanol and centrifuged 10,000 rpm for 10 minutes. The supernatants were pooled and evaporated to dryness in a water bath. Then, the residue was dissolved in 1.0 ml of distilled water. The different aliquots (100, 200 and 300 mg/ml) were pipetted out and the volume made up to 3.0 ml with distilled water in each tube. Then, 0.5 ml of 1 N Folin-Ciocalteau reagent and 2 ml of 20 % sodium carbonate were added, and the tubes were placed in boiling waterbath for one minute. The tubes were cooled and the absorbance read in a spectrophotometer at 650 nm, against a reagent blank. Standard gallic acid solutions (20 %, 0.2-1 ml) corresponding to 2.0 to 10 µg/ml concentrations were also treated as above, and used to prepare a standard curve.

The concentration of phenols in the samples were determined from the standard curve and expressed as µg/ml.

4. Estimation of total flavonoids content

The total flavonoids in the samples was estimated by the method outlined by Cameron, Milton and Allen (1943).

Principle: This test is based on the reaction of flavonoids in the sample with vanillin to produce a coloured product which can be measured spectrophotometrically at 340 nm.

Flavonoids extraction: A volume of 0.5ml each of the samples were first mixed with methanol:water mixture (2:1, that is, 2 ml of methanol and 1 ml of distilled water) and secondly, with the same mixture in the ratio of 1:1 (that is, 1 ml of methanol and 1 ml of distilled water). The mixtures were shaken well and allowed to stand overnight. The supernatants were pooled, the volume measured, and then concentrated on a water bath and used for the assay.

Procedure: A volume of 0.5 ml of the aliquot was pipetted out and evaporated to dryness in water bath. Then, 4.0 ml vanillin reagent (1 % vanillin in 70 % sulphuric acid) was added and the tubes heated in a boiling waterbath for 15 minutes. Varying concentrations (5 – 30 µg/ml) of the standard gallic acid was also treated in the same manner.

The absorbance was read in a spectrophotometer at 340 nm. A standard curve was plotted and the concentration of flavonoids in each sample was extrapolated and expressed as µg/ml sample.

5. Total antioxidant capacity (TAC) assay

The TAC of the samples in was determined by the phosphomolybdate method according to Jayaprakasha, Jena, Negi and Sakariah (2002).

Principle: Phosphomolybdate ion is reduced in the presence of an antioxidant resulting in the formation of a green coloured complex which can be measured spectrophotometrically at 695 nm.

Procedure: An aliquot (30 ml) of different concentrations (100, 200 and 300 mg/ml) of the test samples was mixed with 3 ml of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, 4 mM ammonium molybdate) taken in test tubes. The tubes were capped with aluminium foil and incubated in a boiling waterbath at 95°C for 90 minutes. The reaction mixture was allowed to cool to room temperature and the absorbance of the solution measured at 695 nm against a blank containing 3 ml of reagent solution and methanol in place of sample. The blank was incubated under the same conditions as the test samples. Gallic acid (5-30 µg/ml) was used to prepare a standard curve. The results were expressed as µg/ml.

6. Measurement of superoxide scavenging activity

The superoxide scavenging ability of the samples was assessed by the method of Winterbourn, Hawkins, Brian and Carrell (1975).

Principle: Superoxide radical is produced by photo reduction of riboflavin. Nitroblue tetrazolium (NBT) is reduced to blue formazan by the superoxide anion radical and measured spectrophotometrically at 560 nm.

Procedure: Superoxide anions were generated in the samples that contained in it 3.0 ml of mixture of 0.02 ml of the samples (20 mg), 0.2 ml of EDTA (0.1 M containing 1.5 mg of NaCN), 0.1 ml of NBT (1.5 M), 0.05 ml of riboflavin (0.12 mM) and 2.63 ml of phosphate buffer (0.067M, pH 7.6). The control tubes were also set up where DMSO was added instead of the samples. All the tubes were vortexed and the initial optical density measured at 560 nm in a spectrophotometer. The tubes were illuminated using a fluorescent lamp for 30 minutes. The absorbance was measured again at 560 nm. The difference in absorbance before and after illumination was indicative of superoxide anion scavenging activity, and calculated thus;

Superoxide anion scavenging activity (%) = $[(A_0 – A_1)/A_0] \times 100$ (Equation 3.2)

Where, A_0 - Absorbance of control

A_1 - Absorbance in the presence of sample

7. Hydrogen peroxide scavenging activity assay

The ability of the samples to scavenge hydrogen peroxide was determined by the method of Ruch, Cheng and Klaunig (1989).

Principle: The amount scavenging potential against a molecule is based on intrinsic absorption of H_2O_2 and quantified using a spectrophotometer at 230 nm wavelength.

Procedure: A solution of H_2O_2 (40 mM) was prepared in sodium phosphate buffer (0.1M, pH 7.4). The samples (0.1 ml) of the different concentrations (100, 200, and 300 mg/ml) were added to H_2O_2 solution (0.6 ml) and the total volume made up to 3 ml with distilled water. The reaction mixture was vortexed and after 10 min, the absorbance of the reaction mixture was recorded at 230 nm in a spectrophotometer. A blank solution containing phosphate buffer, without H_2O_2 was prepared. Gallic acid (100 mg/ml) was used as positive control. The extent of H_2O_2 scavenging of the samples was calculated as:

Hydrogen peroxide scavenging activity (%) = $[(A_0 – A_1)/A_0] \times 100$ (Equation 3.3)

Where, A_0 - Absorbance of control

A_1 - Absorbance in the presence of sample

8. Measurement of hydroxyl radical scavenging activity

The extent of hydroxyl radical scavenging from Fenton reaction was quantified using 2'-deoxyribose oxidative degradation as described by Kunchandy and Rao (1990).

Principle: The 2'-deoxyribose degradation product, malondialdehyde, condenses with thiobarbituric acid (TBA) to form a coloured complex which can be quantified spectrophotometrically at 532 nm.

Procedure: The reaction mixture containing 0.1 ml of deoxyribose (2.8 mM), 0.1 ml of $FeCl_3$ (0.1 mM), 0.1 ml of EDTA (0.1 mM), 0.1 ml of H_2O_2, 0.1 ml of ascorbate (1.0 mM), 0.1 ml of KH_2PO_4-KOH buffer (20 mM, pH 7.4) and 20 µl of sample in a final volume of 1.0 ml was incubated at 37^oC for 1 hour. After the incubation, 1.0 ml of 1% TBA was added and heated at 95^oC for 20 minutes to develop colour. After cooling, the thiobarbituric acid reactive substance (TBARS) formed was measured spectrophotometrically at 532 nm against the blank. Gallic acid 100 mg/ml concentration was used as positive control.

Percentage inhibition of hydroxyl radical (%) = $[(A_0 – A_1)/A_0] \times 100$ (Equation 3.4)

Where, A_0 - Absorbance of control, A_1 - Absorbance in the presence of sample

9. DPPH scavenging activity assay

The scavenging ability of the natural antioxidants of the samples towards the stable free radical DPPH was measured using the method of Mensor et al. (2001).

Principle: The antioxidant reacts with stable free radical, DPPH and converts it to 1, 1-Diphenyl -2- picryl hydrazine. A coloured complex is formed which can be measured spectrophotometrically at 518nm.

Procedure: The samples (20 µl) were added to 0.5 ml of 0.1 mM methanolic solution of DPPH (0.3 mM in methanol) and 0.48 ml of methanol. The mixture was left at room temperature for 30 minutes to react. Methanol served as the blank and DPPH in methanol, without the samples, served as the positive control, while butylated hydroxytoluene (BHT) served as reference. After 30 minutes of incubation, the discoloration of the purple colour was measured at 518 nm in a spectrophotometer. The radical scavenging activity was calculated as follows:

Scavenging activity (%) = $[A_{518}(\text{control}) - A_{518}(\text{sample})/A_{518}(\text{control})] \times 100$ (Equation 3.5)

10. ABTS scavenging activity

The antioxidant effect of the samples was studied using ABTS radical cation decolorization assay according to the method described by Shirwaikar, Shirwaikar, Rajendran and Punitha (2006).

Principle: Pre-formed ABTS radical solution, which has a stable blue-green colour reacts with antioxidants in the sample leading to decolorization. The extent of decolorization can be measured spectrophotometrically at 745 nm.

Procedure: ABTS radical cations ($ABTS^{+}$) was produced by reacting ABTS solution (7 mM) with 2.45 mM ammonium persulphate in a ratio of 1:0.5 (that is, 1 ml ABTS solution and 0.5 ml ammonium persulphate). The mixture was allowed to stand in the dark at room temperature for 12 to 16 hours before use. Aliquots (0.5 ml) of the samples were added to 0.3 ml of ABTS radical solution and the final volume made up to 1 ml with ethanol. The absorbance was read at 745 nm in a spectrophotometer. For the control, methanol was used, instead of the test compound. The reference was set up too with gallic acid (100 mg/ml). The per cent inhibition was calculated using the formula:

Inhibition (%) = [(Control – test)/Control] × 100 (Equation 3.6)

Gallic acid was used as control to compare the activities of the samples, except for the DPPH scavenging activity assay.

3.2.3 Experimental animal handling

The rats were acclimatized in different cages, six per cage (standard laboratory metal animal cage) for 14 days. The animals were maintained under standard environmental conditions (12 hour light and 12 hour dark cycle uniform temperature of 28 ± 3 °C). All animals had free access to food and water throughout the period of investigation. All investigations involving the experimental animals were conducted in accordance with the accepted principles for animal care and use according to Naational Institutes of Health (2011).

3.2.4 Induction of benign prostatic hyperplasia

Benign prostatic hyperplasia was induced by administration of 10 mg/kg b.w (body weight) of exogenous testosterone and oestradiol in the ratio of 1:1 intraperitoneally (every 2 days) for four weeks. The steroids were prepared in olive oil as solvent (Mbaka, Ogbonnia, Olarewaju & Duru, 2013).

3.2.5 Animal grouping and treatment

Groups	**Number of rats**	**Treatment for 28 days**
A (NC)	6	Water and feed
B (DC)	6	BPH induced rats + solvent. (Untreated)
C (SC)	6	BPH induced rats + Finasteride at 10 mg/kg bw (standard drug)
D (10 ml/kg bw AM)	6	BPH induced rats + 10 ml/kg bw of soursop juice
E (10 ml/kg bw AC)	6	BPH induced rats + 10 ml/kg bw of onion juice
F (10 ml/kg bw AMAC)	6	BPH induced rats + 1:1 combination of soursop and onion (10 ml/kg bw)
G (20 ml/kg bw AM)	6	BPH induced rats + 20 ml/kg bw of soursop juice
H (20 ml/kg bw AC)	6	BPH induced rats + 20 ml/kg bw of onion juice
I (20 ml/kg bw AMAC)	6	BPH induced rats + 1:1 combination of soursop and onion (20 ml/kg bw)

Key:

NC - Normal control

DC - Disease control

SC - Standard control

AM - *Annona muricata*

AC - *Allium cepa*

AMAC - *Annona muricata* and *Allium cepa*

The treatment was administered orally using gavage tube. At the end of the 28 days treatment, the rats were fasted overnight and euthanized by cervical dislocation and blood was collected into plain sample tubes and allowed to clot. Serum was separated within one hour of blood clotting by centrifugation at 3000 g for 20 minutes. The serum samples were collected using Pasteur pipette and stored at 4 ^{o}C until required for use for the necessary assays. The prostates were carefully excised and rinsed in ice-cold 1.15 % KCl, blotted and weighed using electronic weighing balance (Akanni et al., 2020).

3.2.6 Determination of body weight

The body weights of the rats were recorded before commencement of experiment, and subsequently once a week till sacrificed (Majeed, Bani, Natarajan, Pandey & Naveed, 2017).

3.2.7 Calculation of percentage change in weight

The percentage change in weight was calculated as follows:

Final body weight – Initial body weight x 100/Final body weight (Equation 3.7)

3.2.8 Calculation of relative prostate weight, prostatic index and percentage prostate increase inhibition

Relative Prostate weight (g/kg) = Prostate weight x 1000/Final body weight (Equation 3.8)

Prostate index (mg) = Prostate weight/Body weight

Percentage prostate increase inhibition (%) = 100 – [(T – C)/(B – C) x 100] (Equation 3.9)

Where C, B, and T are the values of the normal control, disease control, and treatment group (Sasidharan, KP, Bhaumik, Kanti Das & Nair, 2022).

3.2.9 Assay of oxidative stress markers

1. Determination of superoxide dismutase activity

This assay was carried out according to the procedure of Das, Samanta and Chainy (2000).

Principle: Superoxide radical is generated by photoreduction of riboflavin and detected by nitrite formation from hydroxylamine hydrochloride at 543 nm.

Procedure: In this assay, 1.4 ml of the reaction mixture comprising 1.1 ml of 50 mM phosphate buffer (pH=7.4), 0.075 ml of 20 mM L-Methionine, 0.4 ml of 1 % (v/v Triton X-100, 0.075 ml of 10 mM hydroxylamaine and 0.1 ml of the sample was incubated at 30 ^{o}C for 5 minutes. This was followed by the addition of 80 µl of 50 µM riboflavin and the tubes were exposed to incandescent light (200 watts lamp) for 10 minutes. The control tube was also set up alongside the test but with the phosphate buffer added instead of the serum. Thereafter, 1 ml of 1 % sulphanilamide and 1 ml of 0.1 % NED was added and the absorbance of the colour formed was measured at 543 nm. One unit of enzyme activity was measured as the amount of SOD capable of inhibiting 50 % of nitrate formation under the assay condition.

The enzyme activity was calculated using: V_0/V-1. (Equation 3.10)

Where, V_0 is the absorbance of the control and V is the absorbance of the sample.

2. Determination of glutathione peroxidase activity

Glutathione peroxidase (GPx) activity in the samples was measured by the method described by Rotruck et al. (1973).

Principle: The decrease in GSH content after incubation of the sample in the presence of H_2O_2 and NaN_3 is measured to determine GPx activity. This stable yellow coloured product formed when Ellman's reagent is added to a sulfhydryl compound is measured spectrophotometrically at 412 nm.

Procedure: The reaction mixture consisting of 0.5 ml of phosphate buffer (0.2 M, pH 7.0 containing 0.4 mM EDTA, and 10 mM sodium azide), 0.2 ml of sample, 0.2 ml of GSH (2 mM) and 0.1 ml of H_2O_2 (0.2 mM) was incubated for 10 min at room temperature along with blank containing all reagents except sample. The reaction was stopped by the addition of 0.5 ml of 10 % TCA (trichloroacetic acid), centrifuged at 4000 rpm for 5 minutes and GSH content in 0.5 ml of supernatant was estimated by adding 4.5 ml of 0.6 mM Ellman's reagent. Mixture was vortexed and the absorbance of mixture read at 412 nm within 15 minutes. Concentration of glutathione was calculated by using standard glutathione of known concentration subjected to the same experimental conditions The activity was expressed as μg of GSH consumed/min/ml.

GPx activity = Amount of glutathione/ml of sample (Equation 3.11)

3. Determination of glutathione-S-transferase activity

Glutathione-S-transferase (GST) activity in samples was measured by the method of Habig, Pabst and Jakoby (1974).

Principle: A conjugation reaction occurs between 1-cloro-2,4-dinitrobenzene and reduced glutathione in the sample. This conjugation is accompanied by an increase in absorbance at 340 nm. The rate of increase is directly proportional to the GST activity.

Procedure: The assay mixture consisted of 1.7 ml of sodium phosphate buffer (0.14 M, pH 6.5), 0.2 ml of GSH (30 mM) and 0.04 ml of sample. Then, 0.06 ml of 0.01 M CDNB dissolved in 50 % ethanol was added, initiating the reaction. Upon addition of CDNB, the increase in absorbance at 340 nm was measured as a function of time.

The activity was calculated by using molar extinction coefficient of CDNB-GSH conjugate as 9.6 $mM^{-1}cm^{-1}$ using; GST activity = absorbance/9.6 $M^{-1}cm^{-1}$(Equation 3.12)

The activity of GST was expressed as μmoles of CDNB-GSH conjugate formed/min/ml.

4. Determination of catalase activity

The catalase enzyme activity in samples was assayed following the procedure of Sinha (1972).

Principle: The method is based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H_2O_2 with the formation of perchromic acid as an unstable intermediate. The chromic acetate produced is measured calorimetrically at 570-610 nm.

Procedure: The sample (0.1 ml) was incubated with H_2O_2 (5 mM, 2.0 ml), in the presence of 2.5 ml 0.01 M phosphate buffer (pH 7.4). 1.0 ml of the mixture was withdrawn and blown into 2 ml of dichromate/acetic acid reagent at 60 seconds intervals. Samples were incubated in boiling water for 15 minutes. After cooling at room temperature, the volume of the reaction mixture was made up to 3 ml with phosphate buffer. The upper layer of the mixture was taken and the absorbance read at 570 nm and the amount of hydrogen peroxide hydrolyzed was calculated for the catalase activity using the mmoles extinction coefficient 40 cm^{-1} and expressed in terms of μmol H_2O_2/min/ml.

Catalase activity = Abs x 1000/40 x ml of sample (Equation 3.13).

5. Determination of malondialdehyde concentration (lipid peroxidation)

Lipid peroxidation in the supernatant fractions is determined spectrophotometrically by assessing the concentration of thiobarbituric acid reactive substances (TBARS) as described by Liu, Edamatsu, Kabuto and Mori (1990).

Principle: MDA reacts with TBA to produce a pink colour chromogen, which is measured spectrophotometrically at 532 nm.

Procedure: Acetic acid 1.5 ml (20 %; pH 3.5), 1.5 ml of thiobarbituric acid (0.8 %) and 0.2 ml of sodium dodecyl sulphate (8.1 %) were added to 0.1 ml of supernatant and heated at 100 ^oC for 60 minutes. After centrifugation at 1200 g for 10 minutes, the organic layer was separated and absorbance (ABS) measured at 532 nm using a spectrophotometer. The results were expressed as malondialdehyde (MDA) concentration, calculated using a molar extinction coefficient of 1.56×105 $M^{-1}cm^{-1}$ and expressed as nanomoles of MDA/ml.

MDA (nmol/ml) = (ABS × 10^6 × 3.3)/(156000 × 0.1 × serum (ml)) (Equation 3.14)

6. Determination of glutathione concentration

Glutathione (reduced) was measured according to the method described by Raja et al. (2007).

Principle: Reduced glutathione (GSH) forms the bulk of non-protein sulfhydryl groups. This method is based on the formation of relatively stable yellow colour when Ellman's reagent (DTNB, 5,5'-dithiobis(2-nitrobenzoic acid)) is added to a sulfhydryl compound. The 2-nitro-5-thiobenzoic acid (TNB^{2-}) is the chromophoric product resulting from the reaction of Ellman's reagent with reduced glutathione which can be measured spectrophotometrically at 412 nm.

Procedure: Equal quantity of sample was mixed with 10 % trichloroacetic acid (1 ml of serum sample and 1 ml of trichloroacetic acid) and centrifuged at 4000 g for 15 minutes to precipitate the proteins. To 0.5 ml of the supernatant, 4.5 ml of 0.6 mM Ellman's reagent was added. Mixture was vortexed and the absorbance of mixture read at 412 nm within 15 minutes. Concentration of glutathione was calculated by using standard glutathione of known concentration subjected to the same experimental conditions.

GSH (mg/ml) = (ABS Test)/(ABS STD) × concentration of standard (Equation 3.15)

7. Determination of total protein concentration

The Biuret method as described by Gornall, Bardawill and David (1949) was employed for the determination of protein concentration using a protein test-kit (Biosystems, U.S.A).

Principle: In an alkaline medium, copper (II) ions or cupric ions in Biuret reagent interact with protein peptide bonds resulting in the formation of a violet coloured complex which can be measured spectrophotometrically at 500 nm.

Procedure: The reagents were brought to room temperature then pipetted into labelled test tubes as follows:

Reagents	Reagent blank (ml)	Standard (ml)	Sample (ml)
Distilled water	0.02	-	-
Standard (66 g/l bovine serum albumin)	-	0.02	-
Sample (Serum)	-	-	0.02
Solution1(Biuret reagent) 6 mmol/l of copper (II) acetate, 12 mmol/l of potassium iodide, 1.15 mmol/l of sodium hydroxide, and detergent	1.0	1.0	1.0

The test tubes were mixed thoroughly and incubated for 30 minutes at room temperature. The absorbance (A) of samples or standard were read against the reagent blank at 500 nm in a spectrophotometer. The total protein concentration in the sample was calculated as follows:

$C_{Sample} = A_{Sample}/A_{Standard} \times C_{Standard}$ (Equation 3.16)

8. Determination of protein carbonyl groups content

The protein carbonyl groups content was measured using the DNPH (2,4-dinitrophenylhydrazine) procedure of Levine et al. (1990).

Principle: Proteins with oxidized lysine residues (carbonyl groups) react with 2,4-dinitrophenyl hydrazine to yield lysine dinitrophenyl hydrazone, a yellow complex that can be measured spectrophotometrically at 368 nm.

Procedure: The purified protein pellets isolated from the incubation mixtures (obtained from total protein determination assay) was redissolved in phosphate buffer (200 mM, pH 7.4) to a final concentration of 2 mg/ml. Aliquots (0.75 ml) of the solution were precipitated with 30 % TCA and centrifuged. The resulting pellets (1.5 mg protein) were treated with 0.5 ml of 10 mM DNPH dissolved in 2 M HCl and allowed to stand for 1 hour at room temperature. TCA (0.5 ml, 20 % w/v) was then added and the samples were centrifuged at 3000 rpm for 10 minutes. The precipitates were washed 3 times with 1 ml of ethanol/ethyl acetate (1:1 v/v), with resuspension, followed by centrifugation. The washed pellets were dissolved overnight in 6 M guanidine solution. Any insoluble material was removed by centrifugation at 3000 rpm for 15 minutes. Carbonyl content was calculated from absorbance readings at 368 nm using a molar absorption coefficient of 22 000 $M^{-1}cm^{-1}$, as follows:

Carbonyl groups (ng/ml BSA) = absorbance/22 000 $M^{-1}cm^{-1}$ (Equation 3.17)

3.2.10 Histological examination of the prostate tissue (Baker, Silver & Pallister, 1997)

Two prostates per group were randomly selected and their dorsolateral lobes were dissected out, and immediately fixed in 10 % formal saline for 7 days for histopathological analysis. The cut-out lobes were placed in tissue cassettes, and then placed in four changes of ascending grades of isopropyl alcohol, (80 %, 90 %, 95 % and absolute) for 2 hours each, to dehydrate the tissue. The tissues were cleared in two changes of xylene for 1 hour each to remove the isopropyl alcohol. The tissues were then transferred to two changes of molten paraffin wax for 1 hour each to eliminate the clearing agent.

Embedding molds were filled with fresh molten paraffin wax and the tissues were placed in them from the cassettes already in wax bath using blunt-nosed forceps. The tissues were orientated until it lay in the deserved plane. The embedding molds were topped-up with molten paraffin wax and allowed to solidify by transferring them to a container of cold water for 10 to 30 minutes. Tissue blocks were detached from the embedding molds, trimmed, and cooled further on ice pack prior to sectioning. Sections were cut using the rotary microtome with the thickness set at 3 µm. The ribbon produced during sectioning were picked and placed in 20% alcohol on a piece of glass, and then floated out in a flotation bath at 40 to 50 ^{o}C. The sections were then picked obliquely using a clean grease free slides.

The sections were dewaxed, hydrated and stained in haematoxylin for 10 minutes, counterstained in 1 % eosin for 1 minute, dehydrated, cleared and mounted in dibutylphthalate polystyrene xylene (DPX) mountant. Slides were viewed, interpreted and photomicrographed using binocular microscope.

3.2.11 Determination of serum dihydrotestosterone level (ELISA) (Brooks, 1984)

Principle: This assay follows the typical competitive binding. Competition occurs between an unlabelled antigen (present in standard, control and test samples) and an enzyme-labelled antigen (conjugate) for a limited number of binding sites on the microwell plate. Unbound materials are removed by washing and decanting procedures. After the washing, the enzyme substrate is added. The enzyme reaction is terminated by adding the stopping solution. The absorbance is read on a microtiter plate reader. The intensity of the colour formed is inversely proportional to the concentration of DHT in the sample. A set of standards is used to plot the standard curve from which the amount of DHT in the sample and controls are read.

Procedure: All reagents were equilibrated to room temperature prior to use. Working solutions of the DHT-HRP conjugate and buffer were prepared, 50 µl of each calibrator, control and the test samples were pipetted into corresponding wells, 100 µl of the conjugate working solution was also pipetted into each of the wells, incubated for 1 hour at room temperature on a plate shaker set to 200 rpm.

The wells were washed 3 times with 300 µl of diluted wash buffer per well, the plate was tapped firmly against absorbent paper to ensure that it is dry. Then, 150 µl of 3,3',5,5'-tetramethylbenzidine (TMB) substrate was pipetted into each well at timed intervals, and incubated for 10 to 15 minutes at room temperature, 50 µl of stop solution was added to each

well at the same timed intervals used before. The plate was read on a microwell reader at 450 nm within 20 minutes of adding stopping solution.

The average optical density of each calibrator duplicate was calculated and a calibrator curve drawn. The mean optical densities of the test samples were determined and read off directly on the calibration curve.

3.2.12 Assay of testosterone (competitive enzyme immunoassay) (Cummings & Wall, 1985)

Principle: Upon mixing biotinylated antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of antibody binding sites. A reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell simultaneously occurs, effecting the separation of the antibody bound fraction after decantation or aspiration. The enzyme activity in the antibody bound fraction is inversely proportional to the concentration of the native antigen in the sample. A dose-response curve can be generated, using several different serum references of known antigen concentration, from which the antigen concentration of an unknown can be determined.

Procedure: All reagents, serum reference calibrators and controls were brought to room temperature before use. The microplates' wells for each serum reference calibrator, control and serum samples to be assayed were formatted in duplicate. A volume of 0.010 ml (10 µl) of the appropriate serum reference calibrator, control or specimen were pipetted into the assigned well. Then, 0.50 ml (100 µl) of the testosterone enzyme reagent was added to all wells. The microplates were swirled gently for 20 to 30 seconds to mix, then covered, and incubated at room temperature for 60 minutes.

The contents of the microplate were then discarded by decantation. The plate was tapped and blotted dry with absorbent paper, 0.350 ml (350 µl) of wash buffer was added, then decanted, tapped and blotted. This step was repeated two additional times for a total of three (3) washes, 0.100 ml (100 µl) of working substrate solution was added to all wells, and not shaken. And then the mixture was incubated at room temperature for 15 minutes, 0.050 ml (50 µl) of stop solution was added to each well and mixed gently for 15 to 20 seconds. All reagents were added in the same order to minimize reaction time differences between wells.

The absorbance in each well was read at 450 nm in a microplate reader. The results were read within 30 minutes of adding the stop solution. A dose response curve was used to ascertain the concentration of testosterone in the samples.

3.2.13 Assay of prolactin (immunoenzymometric sequential assay) (Tietz, 1992)

Principle: In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-prolactin antibody. An antibody-antigen complex forms between the native antigen and the antibody upon mixing monoclonal biotinylated antibody and a serum containing the native antigen. The high affinity reaction of streptavidin and biotinylated antibody simultaneously causes the complex to be deposited to the well.

Decantation or aspiration is done to separate the antibody-antigen bound fraction from unbound antigen after a suitable incubation period. Another antibody that is directed at a different epitope and labelled with an enzyme is added. This results in the occurrence of another interaction forming an enzyme labelled antibody-antigen-biotinylated-antibody complex on the wells' surface. A wash step washes off excess enzyme. A suitable substrate is added, producing colour that is measured with a microplate reader. The enzyme activity on the well is directly proportional to the native antigen concentration. A dose-response curve can be generated, using several different serum references of known antigen concentration, from which the antigen concentration of an unknown can be estimated.

Procedure: All reagents, serum reference calibrators and controls were brought to room temperature before use. The microplates' wells for each serum reference calibrator, control and serum samples to be assayed were formatted in duplicate. Then, 0.025 ml (25 µl) of the appropriate serum reference calibrator, control or specimen were pipetted into the assigned well. 0.100 ml (100 µl) of the prolactin biotin reagent was added into each well. The microplates were swirled gently for 20 to 30 seconds to mix and then covered.

The mixture was incubated for 30 minutes at room temperature. The contents of the microplate were then discarded by decantation. An absorbent paper was used to tap and blot the plate dry. 0.350 ml (350 µl) of wash buffer was added, then decanted, tapped and blotted. This step was repeated two (2) additional times for a total of three (3) washes, and 0.100 ml (100 µl) of prolactin enzyme reagent was added to all wells without shaking. The mixture was incubated at room temperature for thirty (30) minutes. The contents of the microplates were then discarded by decantation. An absorbent

paper was used to tap and blot the plate dry, 0.350 ml (350 µl) of wash buffer was added, then decanted, tapped and blotted. This step was repeated two (2) additional times for a total of three (3) washes, 0.100 ml (100 µl) of working substrate solution was added to all wells, and not shaken. The mixture was incubated at room temperature for 15 minutes, 0.050 ml (50 µl) of stop solution was added to each well and mixed gently for 15 to 20 seconds. All reagents were added in the same order to minimize reaction time differences between wells.

The absorbance in each well was read at 450 nm in a microplate reader. The results were read within 30 minutes of adding the stop solution. A dose response curve was used to ascertain the concentration of prolactin hormone in the test samples.

3.2.14 Assay of prostate specific antigen (PSA) (immunoenzymometric assay) (Wild, 1994)

Principle: In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-PSA antibody. Upon mixing monoclonal biotinylated antibody, the enzyme-labelled antibody and serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. The complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody, simultaneously.

By decantation or aspiration, the antibody-bound fraction is separated from unbound antigen, after equilibrium is attained. The enzyme activity in the antibody-bound fraction is directly proportional to the concentration of the native antigen. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of the unknown can be ascertained.

Procedure: All reagents, serum reference calibrators and controls were brought to room temperature before use. The microplates' wells for each serum reference calibrator, control and serum samples were formatted in duplicate. 0.025 ml (25µl) of the appropriate serum reference calibrator, control or specimen were pipetted into the assigned well. 0.100 ml (100µl) of the PSA enzyme reagent was added into each well. The microplates were swirled gently for 20 to 30 seconds to mix and then covered.

The mixture was incubated for 30 minutes at room temperature. The contents of the microplate were then discarded by decantation. Absorbent paper was used to tap and blot the plate dry. 0.350 ml (350 µl) of wash buffer was added, then decanted, tapped and blotted. This step was repeated two (2) additional times for a total of three (3) washes, and 0.100 ml (100 µl) of working substrate solution was added to all wells, and not shaken. The mixture was incubated at room temperature for fifteen (15) minutes, 0.050 ml (50 µl) of stop solution was added to each well and mixed gently for 15 to 20 seconds. All reagents were added in the same order to minimize reaction time differences between wells.

The absorbance in each well was read at 450 nm in a microplate reader. The results were read within thirty (30) minutes of adding the stop solution. A dose response curve was used to extrapolate the concentration of PSA in unknown specimens.

3.2.15 Determination of interlukin-8 (IL-8) (colorimetric method) (www.abcam.com/ab214030, 2021)

Principle: This kit is based on sandwich enzyme-linked immunosorbent assay technology. It uses an anti-IL-8 antibody pre-coated onto 96-well plates. The biotin conjugated anti-IL-8 antibody is used as detection antibodies. The standards, test samples and biotin conjugated detection antibody are added to the wells and washed with wash buffer. HRP-Streptavidin is added and unbound conjugates washed away with wash buffer. Tetramethylbenzidine (TMB) substrates is used to visualize HRP enzymatic reaction. The TMB is catalyzed by horseradish peroxidase (HRP) to produce a blue colour product that changes into yellow after adding acidic stop solution. The density of the yellow complex is proportional to the IL-8 content of sample captured in plate. Absorbance is read at 450 nm in a microplate reader, and the concentration of IL-8 calculated.

Procedure: All materials and prepared reagents were equilibrated to room temperature prior to use. All reagents, working standards, and samples were prepared according to manufacturer's instruction. To the appropriate wells, 50 µl of all samples or standard were added, 50 µl of the antibody cocktail was also added to each well. The plate was sealed and incubated for 1 hour at room temperature on a plate shaker set to 400 rpm.

Each well was washed with 3 x 350 µl 1 x wash buffer PT by decanting from wells, and dispensing 350 µl 1 x wash buffer PT into each well. Liquid was completely removed at each step as it is essential for good performance. After the last wash, the plate was inverted and

tapped gently against clean paper towels to remove excess liquid. Then, 100 µl of TMB development solution was added to each well and incubated for 10 minutes in the dark on the plate shaker set to 400 rpm, 100 µl of stop solution was added to each well. Colour change from blue to yellow occurred and signal intensity was enhanced to about x 3. The plate was shaken on the plate shaker for 1 minute to mix. The OD was recorded at 450 nm.

The average absorbance value for the blank control (zero) standard was calculated. The average blank control standard absorbance value was subtracted from all other absorbance values, and the standard curve prepared. The concentration of IL-8 in the samples was determined by interpolating the blank control subtracted absorbance values against the standard curve.

3.2.16 Determination of tumor necrosis factor-α (TNF-α) (ELISA) (www.abcam.com/ab181421, 2021)

Principle: This kit employs an affinity tag labelled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is immobilized through immunoaffinity of an anti-tag antibody coating the well. Samples or standards are added to the wells, and followed by the antibody mix. Unbound materials are removed by washing the wells after incubation. TMB development solution is added and catalyzed by HRP during incubation, generating a blue colour. This reaction is stopped by addition of stop solution completing any colour change from blue to yellow. The signal generated is proportional to the amount of bound analyte. The intensity is measured at 450 nm.

Procedure: All materials and prepared reagents were equilibrated to room temperature prior to use. All reagents, working standards, and samples were prepared according to manufacturer's instruction. To the appropriate wells, 50 µl of all samples or standard were added, 50 µl of the antibody cocktail was also added to each well. The plate was sealed and incubated for 1 hour at room temperature on a plate shaker set to 400 rpm.

Each well was washed with 3 x 350 µl 1 x wash buffer PT by decanting from wells and dispensing 350 µl 1 x wash buffer PT into each well. Liquid was completely removed at each step as it is essential for good performance. After the last wash, the plate was inverted and blotted against clean paper towels to remove excess liquid, and 100 µl of stop solution was added to each well. Colour change from blue to yellow occurred and signal intensity was enhanced to about x 3. The plate was shaken on a plate shaker for 1 minute to mix. The OD was recorded at 450 nm.

Serially diluted standards were prepared immediately prior to use to generate the standard curve. The average absorbance value for the blank control (zero) standards was calculated and subtracted from all other absorbance values. A standard curve was prepared. The concentration of TNF-α in the sample was determined by interpolating the blank control subtracted absorbance values against the standard curve.

3.2.17 Determination of TNF-β/Lymphotoxin-α (ELISA) (www.abcam.com/ab229202, 2022)

Principle: This kit employs an affinity tag-labelled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This in turn immobilizes the entire complex (capture antibody/analyte/detector antibody) through immunoaffinity of an anti-tag antibody coating the well. The assay is performed by adding samples or standards to the wells, followed by the antibody mix. The wells are washed after incubation to remove unbound material. During incubation, TMB development solution is added and catalyzed by HRP, blue coloration generates. This reaction is stopped by addition of stop solution completing any colour change from blue to yellow. Signal generated is proportional to the amount of bound analyte. The intensity is measured at 450 nm.

Procedure: All materials and prepared reagents were equilibrated to room temperature prior to use. All reagents, working standards, and samples were prepared according to manufacturer's instruction. To the appropriate wells, 50 µl of all samples or standard was added, 50 µl of the antibody cocktail was also added to each well. The plate was sealed and incubated for 1 hour at room temperature on a plate shaker set to 400 rpm.

Each well was washed with 3 x 350 µl 1 x wash buffer PT by decanting from wells and dispensing 350 µl 1 x wash buffer PT into each well. Wash buffer PT was left in the wells for at least 10 seconds. Liquid was completely removed at each step as it is essential for good performance. After the last wash, the plate was inverted and tapped gently against clean paper towels to remove excess liquid. Then, 100 µl of TMB development solution was added to each well and incubated for 10 minutes in the dark on the plate shaker set to 400 rpm.

After, 100 µl of stop solution was added to each well. Colour changed from blue to yellow and signal intensity was enhanced to about x 3. The plate was shaken on the plate shaker for 1 minute to mix. The OD was recorded at 450 nm.

Serially diluted standards were prepared immediately prior to use to generate the standard curve. The average absorbance value for the blank control (zero) standards was calculated and subtracted from all other absorbance values. A standard curve was prepared. The concentration of TNF-β in the sample was determined by interpolating the blank control subtracted absorbance values against the standard curve.

3.2.18 Determination of expression of PCNA and Caspase-3 in prostate tissue (www.abcam.com, n.d)

These were scored using ABCAM® kit for immunohistochemical staining for formalin-fixed paraffin-embedded (FFPE) tissue sections

Principle: Immunohistochemistry is a method of using antibodies to localize cellular antigens. Primary antibodies bind specifically to epitopes in antigens. Polyvalent conjugated secondary antibodies are added to bind to primary antibodies. The enzyme conjugate in the secondary antibody (HRP) is then used to convert chromogen like DAB (3'3' diamino benzidine) to insoluble coloured products (dark brown).

Procedure:

Deparaffinization/Rehydration: Slides were placed on hot plate for 10 minutes. Sections were incubated in two washes of xylene for 5 minutes each.Followed by incubation in two washes of 100 % ethanol for10 minutes each. The sections were then incubated in two washes of 95 % ethanol for 10 minutes each. Then, they were washed twice in distilled H_2O for 3 minutes.

Antigen unmasking (10 mM sodium citrate buffer pH 6.0): The slides were brought to a boil in citrate buffer pH 6.0 in a microwave oven for 15 minutes and cooled in distilled water.

Staining protocol: Hydrogen peroxide block (100 µl or 2 drops) was added to cover the sections, and incubated for 10 minutes. The slides were then washed 2 times (2 minutes each) in wash buffer, and100 µl of protein block was applied and incubated for 5 minutes at room temperature to block nonspecific background staining. They were washed once for 2 minutes in wash buffer.

One drop (50 µl) of primary antibody was applied, incubated for 40 minutes, washed 4 times in buffer and 50 µl of biotinylated goat anti-mouse IgG (immunoglobulin G) was applied. This was incubated for 10 minutes at room temperature, washed 4 times (each wash for 3 minutes) in wash buffer, 50 µl of streptavidin peroxidase was applied and incubated for 10 minutes at room

temperature. The slides were rinsed 4 times in buffer, 20 µl of DAB chromogen was added to 1 ml of DAB substrate, mixed by swirling and applied 100 µl to tissue. These were incubated for 10 minutes, and rinsed 4 times in wash buffer, 100 µl of filtered haematoxylin was added to cover the section and incubated for 5 minutes. Tissue was rinsed 5 times in tap water. They were dehydrated in ethanol for 2 minutes, allowed to air dry for 1 minute, cleared in xylene for 3 minutes and mounted immediately using dibutylphthalate polystyrene xylene (DPX) mountant. The sections were viewed and, PCNA and Caspase-3 scores taken.

3.2.19 Determination of 5 α-reductase activity (ELISA) (www.mybiosource.com, 2021)

Principle: The microplate in this kit has been pre-coated with an antibody specific to steroid 5 α-reductase 2 (SRD5a2). Standards or samples are then added to the appropriate microplate wells with a biotin-conjugated antibody specific to SRD5a2. Then, avidin conjugated to horseradish peroxidase (HRP) is added to each microplate well and incubated. After, TMB substrate solution is added and only those wells that contain SRD5a2, biotin-conjugated antibody and enzyme-conjugated avidin will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 ± 10 nm. The concentration of SRD5a2 in the samples is determined by comparing the samples' O.D. to the standard curve.

Ttissue homogenates (the sample) were first prepared as follows: Tissues were rinsed in ice-cold phosphate buffered saline (PBS) and weighed before homogenization. They were minced to small pieces and homogenized them in fresh lysis buffer, w:v of 1:20 to 1:50, that is, 1 ml lysis buffer in 50 mg tissue sample on ice. The resulting suspension was sonicated till the solution was clarified. The homogenates were centrifuged at 10,000 g for 5 minutes. The supernatants were collected and assayed immediately.

Procedure: Wells for diluted standard, blank and sample were prepared and labelled. Seven (7) wells were prepared for standard, 1 well for blank. 100 µl each of dilutions of standard, blank and samples were added into the appropriate wells, and covered with the Plate sealer and incubated for 1 hour at 37 ºC. The liquid in each well was removed and not washed. 100 µl of detection reagent A working solution was added to each well, the wells were covered with the plate sealer and incubated for 1 hour at 37 ºC. The solution was decanted and washed with 350 µl of 1 x wash solution to each well using a squirt bottle and allowed to sit for 1 to 2 minutes.

The remaining liquid was removed from all wells completely by snapping the plate onto absorbent paper. It was totally washed 3 times. After the last wash, any remaining wash buffer was removed by decanting. The plate was inverted and blotted against absorbent paper. 100 µl of detection reagent B working solution was added to each well, the wells were covered with the plate sealer and incubated for 30 minutes at 37 ^{o}C. The wash process was repeated for total of 5 times as previously done. Complete removal of liquid was done at each step as it is essential for good performance. 90 µl of Substrate Solution was added to each well and covered with a new plate sealer, incubated for 10 to 20 minutes at 37 ^{o}C, and protected from light. The liquid turned blue by the addition of substrate solution, 50 µl of stop solution was added to each well. The liquid turned yellow at the addition of stop solution. The liquid was mixed by tapping the side of the plate to make the colour change uniform. Any drop of water and fingerprint on the bottom of the plate was removed and confirmed that there is no bubble on the surface of the liquid. Then, the microplate reader was run and measurement conducted at 450 nm immediately.

The duplicate readings for each standard, control, and samples was averaged and the average zero standard optical density subtracted. A standard curve was prepared. Other values were interpolated from the curve.

3.2.20 Inhibitory 5-α reductase activity assay

Inhibitory 5 α-reductase activity assay was carried out using the spectrophotometric method described by Sun, Zheng and Feng (1998).

Principle: This test relies on decomposing NADPH in a mixture containing 5 α-reductase, testosterone and the test compound. NADPH decomposition (proportional to 5 α-reductase activity) is measured spectrophotometrically at 340 nm.

Procedure: Tissue homogenate was prepared using prostate from the normal control. This is because the prostate was not exposed to any form of treatment. The prostate tissue was cut into small pieces and homogenized in 10 ml of medium (20 mM sodium phosphate, pH 6.5, 0.32 M sucrose and 1 mM EDTA). The homogenate was centrifuged for 15 minutes at 4000 rpm. The supernatant was used as enzyme solution.

Standard curve of NADPH was prepared at 340 nm, using concentrations from 2 to 20 µg/ml in methanol. Tubes were set up and labelled as blank, negative control, positive control and test. Reaction mixtures were prepared by placing 3ml of NADPH (22 µM) into the tubes. Then,

1 ml of enzyme solution was added, followed by 4 ml of Tris-HCl buffer (0.5 mM). To all other tubes, except blank, 2 ml of testosterone was added. Then, to the positive control and the test, 2 ml of finasteride (200 nM) and 2 ml of appropriate test sample, respectively, were added. The absorbance readings of the reaction mixtures were read periodically for 5 minutes for a period of 30 minutes. The corresponding concentration of NADPH was calculated from the standard curve as relative testosterone concentration. This concentration was used to determine the residual amount of testosterone in the reaction mixture.

3.3 Product Packaging

The most effective sample, determined from laboratory results, was packaged. The juice was prepared by traditional method of juice making by blending and filtering. The prepared juice was then packaged in clean, well-labelled bottles and capped.

Sensory evaluation and microbiological analysis were carried out on the soursop juice sample before packaging, as follows:

1. Sensory evaluation

After the preparation of the soursop juice, sensory evaluation was carried out. A questionnaire was prepared for collecting sensory evaluation data. A total of 28 individuals volunteered as tasters, given codes 001 to 028. Copies of the questionnaire were distributed to each individual. Each taster was served 10 ml of the soursop juice, and asked to evaluate the colour, taste, mouth feel, aroma and overall acceptability of the juice. The response/acceptance on the questionnaire was checked on a hedonic scale of 5 (Berdos et al., 2020)

Where; Unacceptable - 0
Poor - 1
Satisfactory - 2
Good - 3
Very good - 4
Excellent - 5

2. Microbiological screening

The culture media; nutrient agar (NA), MacConkey agar (MCA), *Salmonella-Shigella* agar (SSA), triple sugar iron agar (TSI), and potato dextrose agar (PDA) were prepared in Erlenmeyer's flasks as follows:

For NA, 2.8 g was dissolved in 100 ml of distilled water. While for MCA, SSA, TSI, and PDA 5.155 g, 6.3 g, 6.462 g, and 3.9, respectively were also dissolved in 100 ml of distilled water.

These were placed in an autoclave to sterilize at 121 ^{o}C for 30 minutes at 15 pounds per square inch (psi). They were then removed and poured (20 ml each) into sterile petri dishes, and allowed to solidify. The plates were then inoculated with the soursop juice sample using spread plate technique as described by Aryal (2022).

A tenfold serial dilution of the juice sample was made using 1 ml of the soursop juice sample in 9 ml of sterile water in a test tube. After the serial dilution, the sample was used to inoculate the plates, using sterile swabsticks to collect the samples using dilutions from 10^{-3} to 10^{-7}. The respective swabsticks were then rubbed all over the surface of the solidified agar. This was carried out aseptically on the NA, MCA, SSA, TSI, and PDA plates. After inoculation, the plates were incubated at 37 °C for 24 hours for the bacteriological media (NA, MCA, SSA) and the TSI, and at 27 °C for 48 hours for the PDA medium. After the periods of incubation, the culture plates were observed for visible growth.

The lowest dilution concentration (10^{-3}) with growth (colonies) was counted using the colony counter. The microbial isolates were thereafter identified using their cultural characteristics. The colony forming units (CFU) was calculated using the formula:

CFU (cfu/ml) = (number of colonies x dilution factor)/volume used (Equation 3.18)

3.4 Data Presentation and Statistical Analysis

Results were presented in tables expressed as mean ± SD (standard deviation). Numerical data obtained from this study were analyzed using one-way analysis of variance (ANOVA) with repeated measures on Statistical Product and Service Solutions (SPSS) version 23. Duncan Multiple Range Test (DMRT) was used to test the differences between means at 0.05 level of significance. Photomicrographs of slides were presented as results of histological examinations.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Results of acute toxicity (LD_{50}) studies

Table 4.1 shows the Results of acute toxicity (LD_{50}) studies of *Allium cepa* bulb and *Annona muricata* pulp juices. No death was recorded from the administration of the juice samples of onion bulb and soursop pulp. Also, no behavioural changes were observed in any of the animals even at the highest concentration of 5,000 mg/kg bw (Table 4.1).

Table 4.1: Results of acute toxicity (LD_{50}) studies of onion bulb and soursop pulp juices

Sample	Phase	Group	Number of mice	Mortality	Behavioural changes
Onion bulb juice	I	I	3	0	None
		II	3	0	None
		III	3	0	None
	II	IV	3	0	None
		V	3	0	None
		VI	3	0	None
Soursop pulp juice	I	I	3	0	None
		II	3	0	None
		III	3	0	None
	II	IV	3	0	None
		V	3	0	None
		VI	3	0	None

4.1.2 Ferric reducing antioxidant property of onion bulb and soursop pulp juices

Table 4.2 shows the ferric reducing antioxidant property of *Allium cepa* bulb and *Annona muricata* pulp juices. The ferric reducing antioxidant property for the juices increased in a dose-dependent manner and were significantly (p<0.05) lower when compared to the reference. Onion juice had a higher FRAP activity than soursop juice (Table 4.2).

Table 4.2: Ferric reducing antioxidant property of *Allium cepa* bulb and *Annona muricata* pulp juices

Sample concentration (mg/ml)	Antioxidant activity of onion juice (µg/ml)	Antioxidant activity of soursop juice (µg/ml)
100	2.63 ± 0.12^{a}	1.67 ± 0.04^{a}
200	2.71 ± 0.04^{a}	1.80 ± 0.12^{b}
300	2.76 ± 0.03^{a}	2.26 ± 0.00^{c}
Reference (gallic acid)	3.59 ± 0.02^{b}	3.59 ± 0.02^{d}

Values are represented as mean ± standard deviation of triplicate determinations. Values on the same column bearing different superscript letters are significantly different (p<0.05).

4.1.3 Nitric oxide scavenging property of onion bulb and soursop pulp juices

Table 4.3 shows the nitric oxide property of *Allium cepa* and *Annona muricata* pulp juices. The nitric oxide scavenging activity for the samples increased in a dose-dependent manner, and were not statistically not significant ($p>0.05$). However, these activities for the samples were significantly ($p<0.05$) lower than the reference compound (Table 4.3). The two juices seem to have similar NO scavenging property.

Table 4.3: Nitric oxide scavenging property of *Allium cepa* bulb and *Annona muricata* pulp juices

Sample concentration (mg/ml)	Antioxidant activity of soursop juice (µg/ml)	Antioxidant activity of onion juice (µg/ml)
100	2.39 ± 0.02^{a}	2.29 ± 0.00^{a}
200	2.52 ± 0.03^{a}	2.32 ± 0.01^{a}
300	2.53 ± 0.41^{a}	2.46 ± 0.31^{a}
Reference (gallic acid)	3.44 ± 0.03^{b}	3.44 ± 0.03^{b}

Values are represented as mean ± standard deviation of triplicate determinations. Values on the same column bearing different superscript letters are significantly different ($p<0.05$).

4.1.4 Total phenol content of onion bulb and soursop pulp juices

Table 4.4 shows the total phenol content of *Allium cepa* bulb and *Annona muricata* pulp juices. The total phenol content for the juices increased in a dose-dependent manner and were significantly (p<0.05) lower when compared to the reference. Soursop juice had a higher total phenol content than soursop juice (Table 4.4).

Table 4.4: Total phenol content of *Allium cepa* bulb and *Annona muricata* pulp juices

Sample concentration (mg/ml)	Concentration in soursop juice (µg/ml)	Concentration in onion juice (µg/ml)
100	2.99 ± 0.01^{a}	1.99 ± 0.11^{a}
200	4.87 ± 0.17^{b}	2.65 ± 0.04^{b}
300	6.31 ± 0.02^{c}	3.52 ± 0.09^{c}
Reference (gallic acid)	9.67 ± 0.08^{d}	9.67 ± 0.08^{d}

Values are represented as mean ± standard deviation of triplicate determinations. Values on the same column bearing different superscript letters are significantly different (p<0.05).

4.1.5 Total flavonoids content of onion bulb and soursop pulp juices

Table 4.5 shows the total flavonoids content of *Allium cepa* bulb and *Annona muricata* pulp juices. The total flavonoids content for onion juice increased in a dose-dependent manner, and not significantly (p>0.05) different from the reference compound. The total flavonoids content of soursop juice reduced dose-dependently, and significantly lower. However, significantly (p<0.05) lower when compared to the reference. Onion juice had a higher total flavonoids content than soursop juice (Table 4.5).

Table 4.5: Total flavonoids content of *Allium cepa* bulb and *Annona muricata* pulp juices

Sample concentration (mg/ml)	Concentration in soursop juice (µg/ml)	Concentration in onion juice (µg/ml)
100	58.36 ± 0.12^{a}	58.83 ± 0.07^{a}
200	58.21 ± 0.02^{a}	58.88 ± 0.02^{a}
300	58.17 ± 0.02^{a}	59.19 ± 0.58^{a}
Reference (gallic acid)	59.33 ± 0.19^{b}	59.33 ± 0.19^{a}

Values are represented as mean ± standard deviation of triplicate determinations. Values on the same column bearing different superscript letters are significantly different (p<0.05).

4.1.6 Total antioxidant capacity of onion bulb and soursop pulp juices

Table 4.6 shows the total antioxidant capacity of *Allium cepa* bulb and *Annona muricata* pulp juices. The total antioxidant capacity of both juices increased dose-dependently. The total antioxidant capacity of onion juice were significantly (p<0.05) lower than the reference compound, while the that of soursop juice 300 mg/ml concentration was significantly (p<0.05) higher than the reference. Soursop juice had a higher total flavonoids content than onion juice (Table 4.6).

Table 4.6: Total antioxidant capacity of *Allium cepa* bulb and *Annona muricata* pulp juices

Sample concentration (mg/ml)	Antioxidant activity of soursop juice (µg/ml)	Antioxidant activity of onion juice (µg/ml)
100	3.98 ± 0.33^{a}	2.60 ± 0.15^{a}
200	4.53 ± 0.29^{b}	3.88 ± 0.08^{b}
300	13.09 ± 0.25^{d}	8.24 ± 0.06^{c}
Reference (gallic acid)	8.83 ± 0.05^{c}	8.83 ± 0.05^{d}

Values are represented as mean ± standard deviation of triplicate determinations. Values on the same column bearing different superscript letters are significantly different (p<0.05).

4.1.7 Superoxide scavenging property of onion bulb and soursop pulp juices

Table 4.7 shows the superoxide scavenging property of *Allium cepa* bulb and *Annona muricata* pulp juices. Values obtained for the samples significantly increased with increase in concentration, but were significantly (p<0.05) lower than the reference (Table 4.7).

Table 4.7: Superoxide scavenging property of *Allium cepa* bulb and *Annona muricata* pulp juices

Sample concentration (mg/ml)	Percentage inhibition by soursop juice (%)	Percentage inhibition by onion juice (%)
100	54.69 ± 0.93^{a}	52.85 ± 0.69^{a}
200	57.66 ± 0.62^{b}	58.13 ± 0.67^{b}
300	61.83 ± 0.55^{c}	63.72 ± 0.87^{c}
Reference (gallic acid)	70.31 ± 0.52^{d}	70.31 ± 0.52^{d}

Values are represented as mean ± standard deviation of triplicate determinations. Values on the same column bearing different superscript letters are significantly different (p<0.05).

4.1.8 Hydrogen peroxide scavenging property of onion bulb and soursop pulp juices

Table 4.8 shows the hydrogen peroxide scavenging property of *Allium cepa* bulb and *Annona muricata* pulp juices. Values obtained for the samples reduced with increasing concentration. For onion juice, the values were significantly (p<0.05) different from each other. All values obtained for the juices were significantly (p<0.05) lower than the reference (Table 4.8).

Table 4.8: Hydrogen peroxide scavenging property of *Allium cepa* bulb and *Annona muricata* pulp juices

Sample concentration (mg/ml)	Percentage inhibition by soursop juice (%)	Percentage inhibition by onion juice (%)
100	47.72 ± 0.00^{a}	47.24 ± 0.03^{c}
200	47.54 ± 0.03^{ab}	46.54 ± 0.03^{b}
300	47.44 ± 0.03^{b}	46.21 ± 0.05^{a}
Reference (gallic acid)	60.76 ± 0.20^{c}	60.70 ± 0.23^{d}

Values are represented as mean ± standard deviation of triplicate determinations. Values on the same column bearing different superscript letters are significantly different (p<0.05).

4.1.9 Hydroxyl radical scavenging property of onion bulb and soursop pulp juices

Table 4.9 shows the hydroxyl radical scavenging property of *Allium cepa* bulb and *Annona muricata* pulp juices. Values obtained for the samples significantly reduced as the concentration increased, and were also significantly (p<0.05) lower than the reference (Table 4.9).

Table 4.9: Hydroxyl radical scavenging property of *Allium cepa* bulb and *Annona muricata* pulp juices

Sample concentration (mg/ml)	Percentage inhibition by soursop juice (%)	Percentage inhibition by onion juice (%)
100	30.46 ± 1.36^{c}	65.94 ± 0.64^{c}
200	18.33 ± 0.46^{b}	42.44 ± 0.32^{b}
300	14.55 ± 1.21^{a}	41.16 ± 0.32^{a}
Standard (gallic acid)	80.45 ± 0.76^{d}	80.45 ± 0.76^{d}

Values are represented as mean ± standard deviation of triplicate determinations. Values on the same column bearing different superscript letters are significantly different (p<0.05).

4.1.10 DPPH scavenging property of onion bulb and soursop pulp juices

Table 4.10 shows the DPPH scavenging property of *Allium cepa* bulb and *Annona muricata* pulp juices. The results obtained increased as the concentration increased, but were lower than the reference. Only onion juice at 300 mg/ml concentration showed value statistically similar to the reference, the rest were significantly (p<0.05) lower (Table 4.10).

Table 4.10: DPPH scavenging activity of *Allium cepa* bulb and *Annona muricata* pulp juices

Sample concentration (mg/ml)	Percentage scavenging activity by soursop juice (%)	Percentage scavenging activity by onion juice (%)
100	85.87 ± 0.14^{a}	85.41 ± 0.72^{a}
200	87.21 ± 0.17^{b}	93.09 ± 0.86^{b}
300	95.23 ± 0.53^{c}	97.77 ± 0.25^{c}
Reference (BHT)	98.24 ± 0.00^{d}	98.24 ± 0.00^{c}

Values are represented as mean ± standard deviation of triplicate determinations. Values on the same column bearing different superscript letters are significantly different (p<0.05).

4.1.11 ABTS scavenging property of onion bulb and soursop pulp juices

Table 4.11 shows the ABTS scavenging property of *Allium cepa* bulb and *Annona muricata* pulp juices. Values obtained for soursop juice samples were significantly (p<0.05) less than that of the reference, while the values obtained for onion juice samples were significantly (p<0.05) higher than the reference. Also, the values increased with increase in concentration (Table 4.11).

Table 4.11: ABTS scavenging activity of *Allium cepa* bulb and *Annona muricata* pulp juices

Sample concentration (mg/ml)	Percentage scavenging activity by Soursop juice (%)	Percentage scavenging activity by Onion juice (%)
100	55.29 ± 0.11^{a}	67.13 ± 0.11^{b}
200	57.80 ± 0.29^{b}	68.18 ± 0.11^{c}
300	59.62 ± 0.21^{c}	71.14 ± 0.11^{d}
Reference (gallic acid)	61.31 ± 0.00^{d}	61.31 ± 0.00^{a}

Values are represented as mean ± standard deviation of triplicate determinations. Values on the same column bearing different superscript letters are significantly different (p<0.05).

4.1.12 Effect of onion bulb and soursop pulp juices on body weight of albino rats with testosterone and oestradiol-induced BPH

Table 4.12 presents the effect of *Allium cepa* bulb and *Annona muricata* pulp juices onbody weight of albino rats with testosterone and oestradiol-induced BPH. There was increase in the body weight of all the groups at day 7 (week 1) of treatment. A drop in body weight was observed in the disease control, and the groups treated with high dose of onion juice and combined juice at day 14 (week 2). A further drop in body weight in these groups and the group treated with high dose of soursop was observed at day 21 (week 3). While at week 4, there was an increase in the body weight of all the groups, except for the group treated with high dose of onion juice where a further decrease in body weight was observed. Steady increase in body weight was observed in the normal control, standard control, and the low dose groups throughout the weeks (Table 4.12).

Table 4.12: Effect of *Allium cepa* bulb and *Annona muricata* pulp juices on body weight of albino rats with testosterone and oestradiol-induced BPH

Group	Body weight per week (g)				
	Initial weight	Week 1	Week 2	Week 3	Final weight (Wk 4)
NC	177.13 ± 19.13	196.31 ± 22.65	218.15 ± 26.18	235.18 ± 31.03	244.60 ± 47.56
DC	198.56 ± 18.26	200.04 ± 17.03	197.92 ± 13.50	189.68 ± 20.61	199.84 ± 15.48
SC	159.37 ± 1.98	166.52 ± 3.71	167.23 ± 8.40	175.54 ± 8.38	185.41 ± 9.70
10 ml/kg AM	148.47 ± 6.80	158.13 ± 5.21	162.93 ± 3.20	168.55 ± 2.42	176.42 ± 4.99
10 ml/kg AC	140.26 ± 17.45	153.75 ± 17.52	161.30 ± 16.61	162.23 ± 18.73	165.46 ± 20.21
10 ml/kg AMAC	151.68 ± 13.57	159.98 ± 19.32	167.43 ± 13.63	170.65 ± 11.97	176.06 ± 15.92
20 ml/kg AM	163.18 ± 9.27	171.36 ± 5.73	172.70 ± 7.93	168.80 ± 10.42	165.88 ± 16.48
20 ml/kg AC	179.80 ± 26.23	188.88 ± 22.33	185.65 ± 14.00	180.57 ± 3.00	189.69 ± 9.98
20 ml/kg AMAC	177.95 ± 17.18	184.29 ± 12.48	182.58 ± 13.84	182.13 ± 10.75	187.81 ± 7.26

Values are represented as mean ± standard deviation of triplicate determinations.

4.1.13 Effect of onion bulb and soursop pulp juices on percentage body weight and average percentage body weight change of albino rats with testosterone and oestradiol-induced BPH

Table 4.13 presents the effect of *Allium cepa* bulb and *Annona muricata* pulp juices on percentage body weight change and average percentage body weight change of albino rats with testosterone and oestradiol-induced BPH. The soursop juice at low dose (10 ml/kg AM) showed the highest average percentage weight change among the treatment groups and standard control. Generally, the values obtained at low doses were higher than those of the high doses, and were not significantly (p>0.05) different from the standard control. Average percentage body weight change of all the test groups were significantly (p<0.05) lower than the normal control (Table 4.13).

Table 4.13: Effect of *Allium cepa* bulb and *Annona muricata* pulp juices on percentage body weight change and average percentage body weight change of albino rats with testosterone and oestradiol-induced BPH

Group	Percentage body weight change per week (%)				Average percentage body weight change (%)
	Week 1	Week 2	Week 3	Week 4	
NC	9.70 ± 3.30^{c}	9.98 ± 1.18^{b}	7.13 ± 2.19^{d}	3.10 ± 6.47^{ab}	7.48 ± 2.00^{c}
DC	0.78 ± 0.69^{a}	-1.00 ± 1.75^{a}	-4.67 ± 5.22^{a}	5.23 ± 3.28^{b}	0.08 ± 0.70^{a}
SC	4.24 ± 3.33^{abc}	0.18 ± 7.39^{a}	4.73 ± 1.59^{cd}	5.30 ± 1.51^{b}	3.61 ± 1.14^{b}
10 ml/kg AM	6.13 ± 1.67^{abc}	2.96 ± 1.94^{a}	3.32 ± 2.42^{bcd}	4.40 ± 3.41^{ab}	4.20 ± 1.70^{b}
10 ml/kg AC	8.85 ± 1.04^{bc}	4.75 ± 1.16^{ab}	0.47 ± 1.56^{abcd}	1.89 ± 0.77^{ab}	3.99 ± 0.28^{b}
10 ml/kg AMAC	4.88 ± 5.22^{abc}	4.62 ± 4.74^{ab}	1.92 ± 2.70^{abcd}	2.93 ± 3.33^{ab}	3.59 ± 2.81^{b}
20 ml/kg AM	4.83 ± 2.27^{abc}	0.73 ± 1.27^{a}	-2.44 ± 4.70^{ab}	-2.06 ± 5.18^{a}	0.27 ± 1.41^{a}
20 ml/kg AC	4.99 ± 3.05^{abc}	-1.54 ± 4.71^{a}	-2.75 ± 6.01^{ab}	4.69 ± 3.41^{ab}	1.35 ± 2.33^{ab}
20 ml/kg AMAC	3.54 ± 3.54^{ab}	-0.98 ± 1.27^{a}	-0.18 ± 1.86^{abc}	3.07 ± 2.54^{ab}	1.36 ± 1.68^{ab}

Values are represented as mean ± standard deviation of triplicate determinations. Values on the same column bearing different superscript letters are significantly different (p<0.05).

4.1.14 Effect of onion bulb and soursop pulp juices on prostate weight (PW), prostate index (PI), relative prostate weight (RPW) and percentage prostate increase inhibition (PPII) of albino rats with testosterone and oestradiol-induced BPH

Table 4.14 shows the effect of *Allium cepa* bulb and *Annona muricata* pulp juices on prostate weight, prostate index, relative prostate weight and percentage prostate increase inhibition of albino rats with testosterone and oestradiol-induced BPH. The prostate weight, prostate index and relative prostate weight of all test groups were significantly (p<0.05) higher than the normal control, and statistically similar to the standard control. The PPII values for all the test groups at low doses were higher than at high doses, with the highest observed in low dose onion juice, followed by low dose soursop juice. Thus, at low doses, the extracts inhibited prostate increase better than at high dose (Table 4.14).

Table 4.14: Effect of *Allium cepa* bulb and *Annona muricata* pulp juices on prostate weight, prostate index, relative prostate weight and percentage prostate increase inhibition of albino rats with testosterone and oestradiol-induced BPH

Group	Prostate weight (g)	Prostate index (mg x 10^{-2})	Relative prostate weight (g/kg)	Percentage prostate increase inhibition (%)
NC	2.18 ± 0.26^{a}	0.93 ± 0.26^{a}	9.26 ± 2.55^{a}	-
DC	3.31 ± 0.35^{c}	1.66 ± 0.12^{bc}	16.58 ± 1.23^{bc}	-
SC	2.53 ± 0.21^{ab}	1.37 ± 0.16^{b}	13.69 ± 1.55^{b}	69.32 ± 40.15^{b}
10 ml/kg AM	2.85 ± 0.66^{bc}	1.62 ± 0.39^{bc}	16.18 ± 3.87^{bc}	41.00 ± 35.33^{ab}
10 ml/kg AC	2.77 ± 0.13^{abc}	1.69 ± 0.16^{bc}	16.87 ± 1.56^{bc}	48.08 ± 24.44^{ab}
10 ml/kg AMAC	2.98 ± 0.07^{bc}	1.70 ± 0.19^{bc}	17.02 ± 1.89^{bc}	29.79 ± 23.15^{ab}
20 ml/kg AM	3.25 ± 0.22^{c}	1.96 ± 0.07^{c}	19.64 ± 0.72^{c}	5.61 ± 28.81^{a}
20 ml/kg AC	2.95 ± 0.58^{bc}	1.55 ± 0.23^{b}	15.46 ± 2.26^{b}	32.45 ± 34.25^{ab}
20 ml/kg AMAC	3.00 ± 0.07^{bc}	1.60 ± 0.10^{bc}	16.00 ± 0.99^{bc}	27.73 ± 24.50^{ab}

Values are represented as mean ± standard deviation of triplicate determinations. Values on the same column bearing different superscript letters are significantly different (p<0.05).

4.1.15 Effect of onion bulb and soursop pulp juices on SOD, GPx, GST and CAT activities of albino rats with testosterone and oestradiol-induced BPH

Table 4.15 shows the effect of *Allium cepa* bulb and *Annona muricata* pulp juices on SOD, GPx, GST and CAT activities of albino rats with testosterone and oestradiol-induced BPH. among the test groups, the highest SOD and GPx activities were observed in low dose soursop juice, while for GST and CAT the highest activities were observed low dose combined juice. The GSH concentration in low and high-dose combination; GST activity in all the test groups; GPx activity in low dose soursop juice, low dose combination and high dose combination, compared well with the standard control. While SOD activity for all test groups were significantly (p<0.05) lower than the standard control (Table 4.15).

Table 4.15: Effect of *Allium cepa* bulb and *Annona muricata* pulp juices on SOD, GSH, GPX and GST activities of albino rats with testosterone and oestradiol-induced BPH

Group	SOD (IU/ml)	GPx (µgGPx/min/ml)	GST (µmol GSH-CDNB /min/ml x 10^6)	Catalase (µm H_2O_2/min/ml)
NC	106.33 ± 6.06^{e}	3.44 ± 0.05^{e}	16.05 ± 2.72^{c}	16.94 ± 3.32^{bc}
DC	45.52 ± 3.66^{a}	1.34 ± 0.02^{a}	2.20 ± 0.48^{a}	8.37 ± 1.90^{a}
SC	100.10 ± 0.79^{e}	2.66 ± 0.04^{d}	6.52 ± 0.22^{b}	17.52 ± 1.41^{bc}
10 ml/kg AM	91.48 ± 2.57^{d}	2.81 ± 0.03^{d}	7.43 ± 0.80^{b}	20.23 ± 3.36^{cd}
10 ml/kg AC	58.14 ± 2.43^{b}	1.80 ± 0.03^{b}	7.03 ± 0.36^{b}	25.95 ± 5.69^{de}
10 ml/kg AMAC	76.41 ± 2.45^{c}	2.60 ± 0.25^{d}	9.48 ± 3.58^{b}	37.83 ± 4.27^{g}
20 ml/kg AM	79.39 ± 2.41^{c}	2.27 ± 0.02^{c}	6.44 ± 0.26^{b}	13.61 ± 4.27^{ab}
20 ml/kg AC	64.18 ± 3.91^{b}	2.14 ± 0.07^{c}	7.73 ± 1.86^{b}	30.28 ± 2.05^{ef}
20 ml/kg AMAC	60.87 ± 5.51^{b}	2.70 ± 0.25^{d}	7.35 ± 2.87^{b}	33.86 ± 1.29^{fg}

Values are represented as mean ± standard deviation of triplicate determinations. Values on the same column bearing different superscript letters are significantly different (p<0.05).

4.1.16 Effect of onion bulb and soursop pulp juices on MDA, GSH, total protein and PCO groups concentrations of albino rats with testosterone and oestradiol-induced BPH

Table 4.16 presents the effect of *Allium cepa* bulb and *Annona muricata* pulp juices on catalase activity, malondialdehyde, total protein and protein carbonyl groups concentrations of albino rats with testosterone and oestradiol-induced BPH. The extracts significantly (p<0.05) increased GSH concentration significantly (p<0.05), while significantly (p<0.05) reducing the MDA, total protein, and protein carbonyl groups when compared with the disease control (Table 4.16).

Table 4.16: Effect of *Allium cepa* bulb and *Annona muricata* pulp juices on MDA, GSH, total protein and protein carbonyl groups concentrations of albino rats with testosterone and oestradiol-induced BPH

Group	Lipid peroxidation/ MDA (nmol/ml)	GSH (mg/l)	Total protein (mg/ml)	Protein carbonyl groups (ng/ml BSA)
NC	1.80 ± 0.07^{c}	79.10 ± 6.52^{f}	12.69 ± 0.00^{a}	2.21 ± 0.22^{abc}
DC	2.49 ± 0.33^{d}	31.56 ± 0.26^{a}	33.33 ± 0.51^{f}	4.37 ± 1.48^{d}
SC	1.40 ± 0.11^{b}	62.08 ± 0.06^{d}	16.83 ± 0.09^{bc}	2.64 ± 0.05^{bc}
10 ml/kg AM	1.60 ± 0.00^{c}	51.49 ± 0.41^{c}	20.05 ± 0.26^{d}	2.23 ± 0.07^{abc}
10 ml/kg AC	0.97 ± 0.03^{a}	39.71 ± 2.35^{b}	25.21 ± 0.17^{e}	1.44 ± 0.03^{a}
10 ml/kg AMAC	1.28 ± 0.11^{b}	58.91 ± 7.56^{cd}	17.60 ± 1.53^{c}	3.03 ± 0.62^{bc}
20 ml/kg AM	1.37 ± 0.00^{b}	69.59 ± 4.78^{e}	15.82 ± 0.09^{b}	1.92 ± 0.12^{ab}
20 ml/kg AC	1.67 ± 0.02^{c}	51.63 ± 0.67^{c}	19.79 ± 0.51^{d}	2.33 ± 0.13^{abc}
20 ml/kg AMAC	1.61 ± 0.30^{c}	61.56 ± 5.64^{d}	16.75 ± 1.35^{bc}	3.21 ± 0.62^{c}

Values are represented as mean ± standard deviation of triplicate determinations. Values on the same column bearing different superscript letters are significantly different (p<0.05).

4.1.17 Effect of onion bulb and soursop pulp juices on the histology of the prostate of albino rats with testosterone and oestradiol-induced BPH

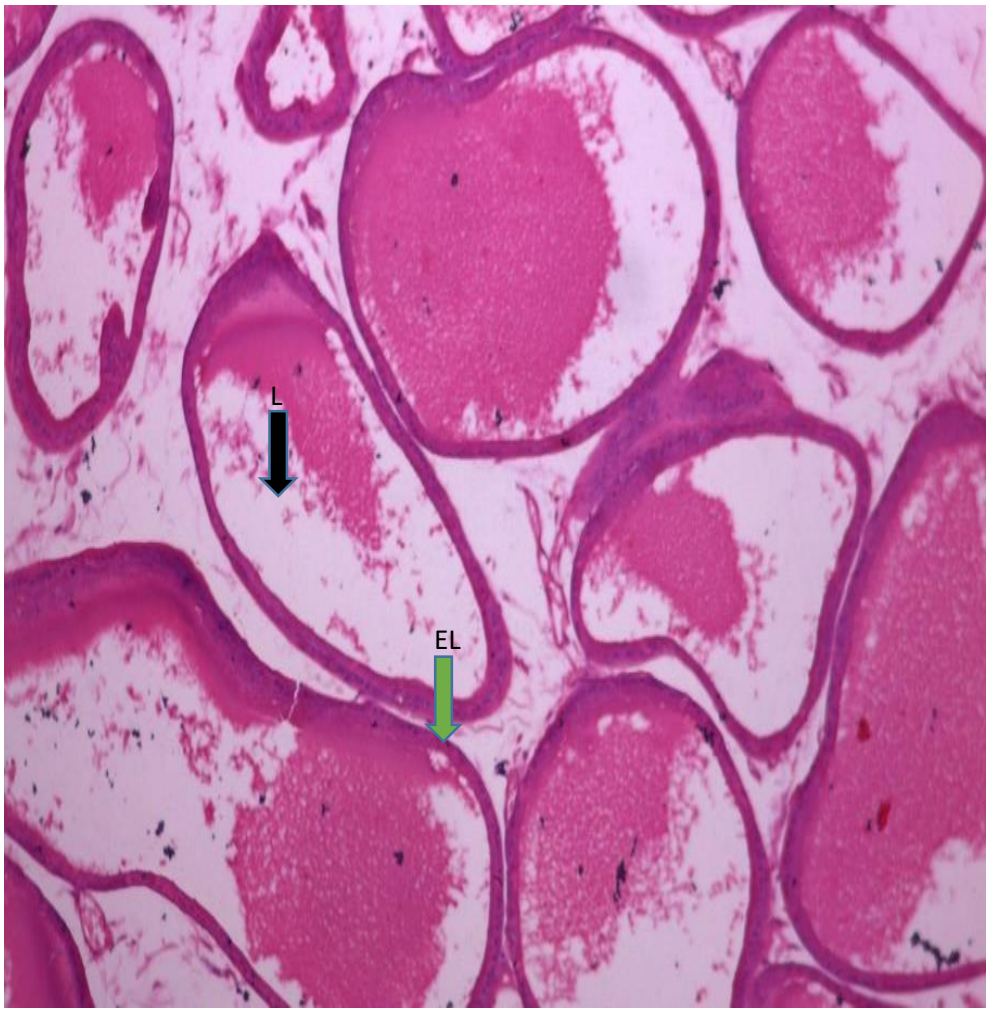

Plate 4.1: Histological section of the prostate from the normal control group. Histological section showed glands with overall features appearing normal. Epithelial lining (EL) thickness was normal. Fibromuscular tissue and lumen (L) appeared normal (H & E stain x400).

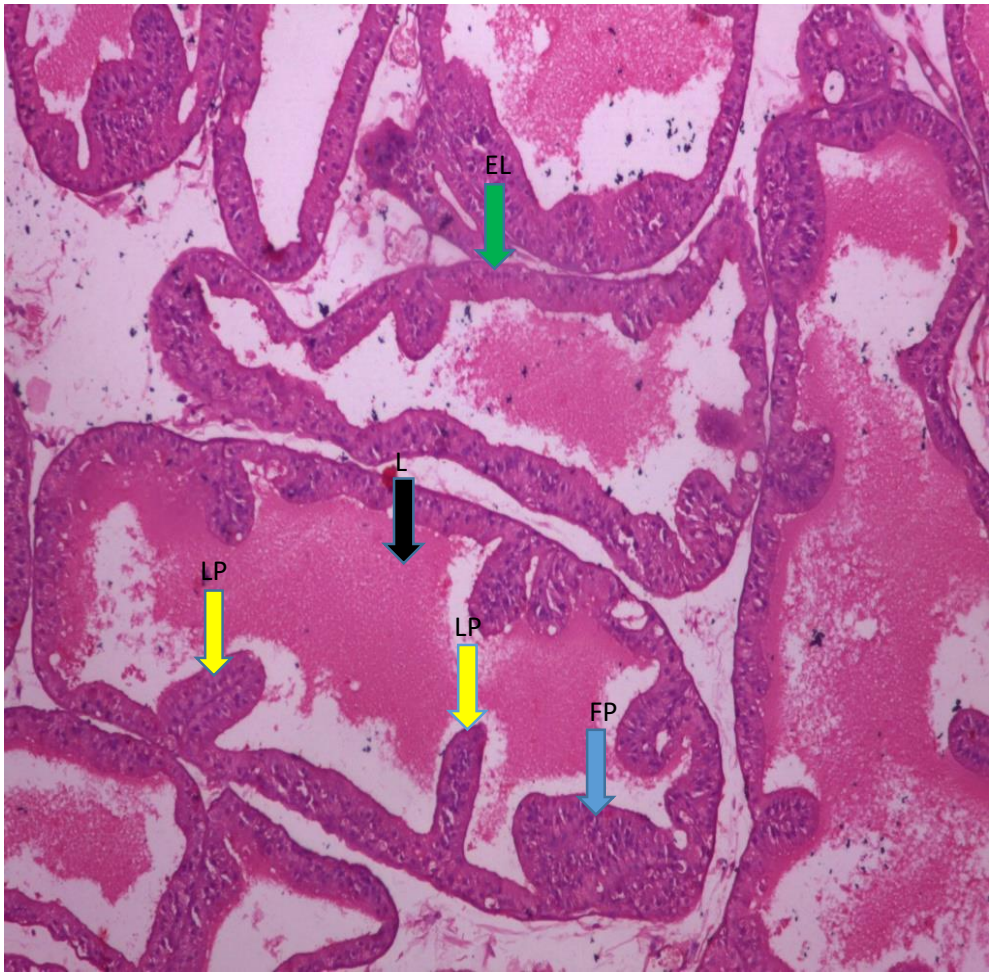

Plate 4.2: Histological section of the prostate from the disease control group. Histological section showed glands with marked increase in thickness of the epithelial lining (EL). There were epithelial/luminal projections (LP) and focal proliferations (FP) causing narrowing of the lumen (L). The fibromuscular tissue stroma was scanty (H & E stain x400).

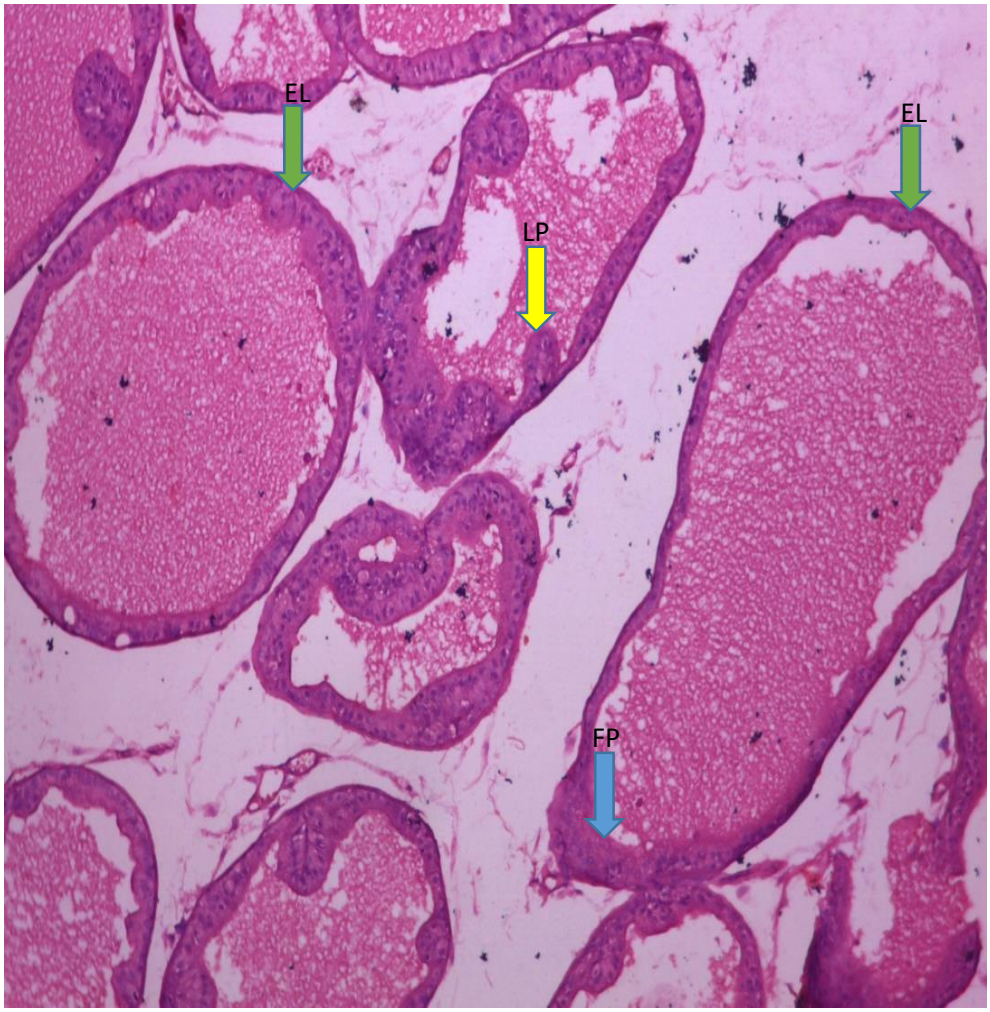

Plate 4.3: Histological section of the prostate from the standard control group. Histological section showed single prostatic glands with fewer epithelial/luminal projections (LP), and focal proliferations (FP). There was mild increase in thickness of the epithelial lining (EL) (H & E stain x400).

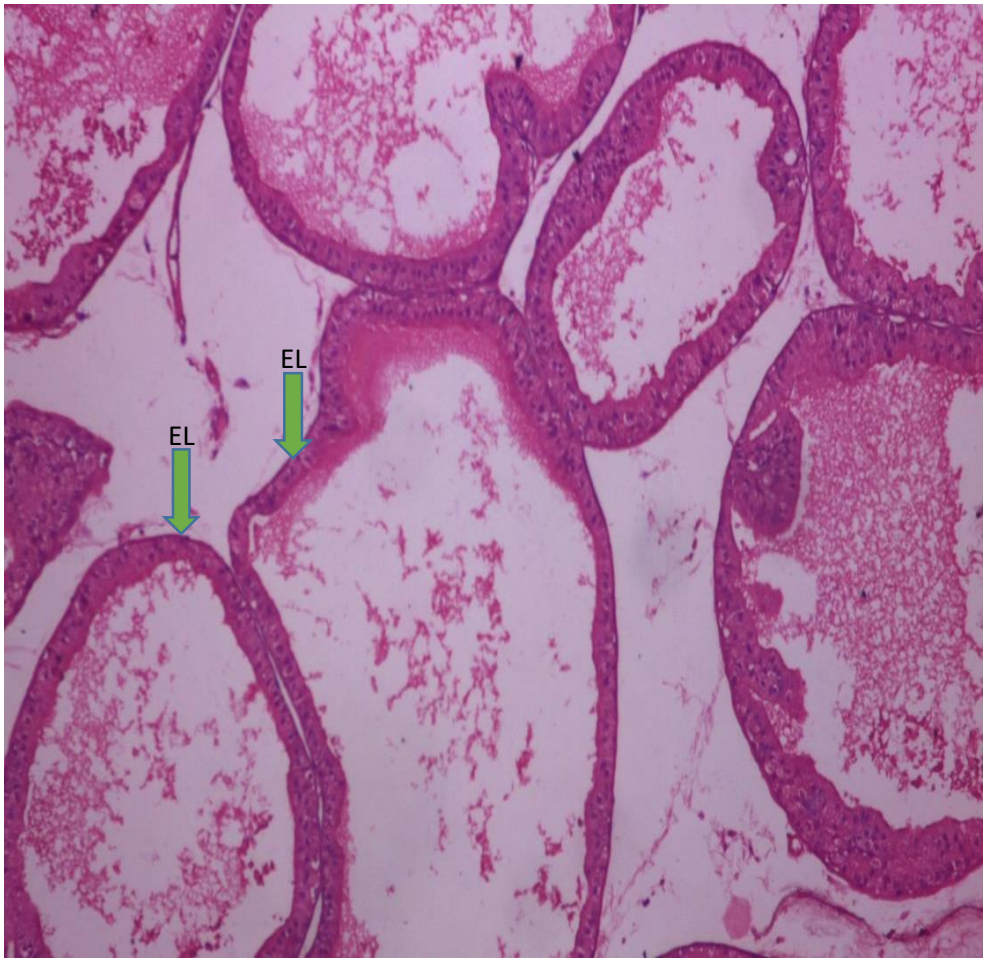

Plate 4.4: Histological section of the prostate from the group treated with 10 ml/kg bw soursop juice. Histological section showed single prostatic glands with epithelial lining (EL) thickness that appeared less remarkable. There was less focal proliferation (H & E stain x400).

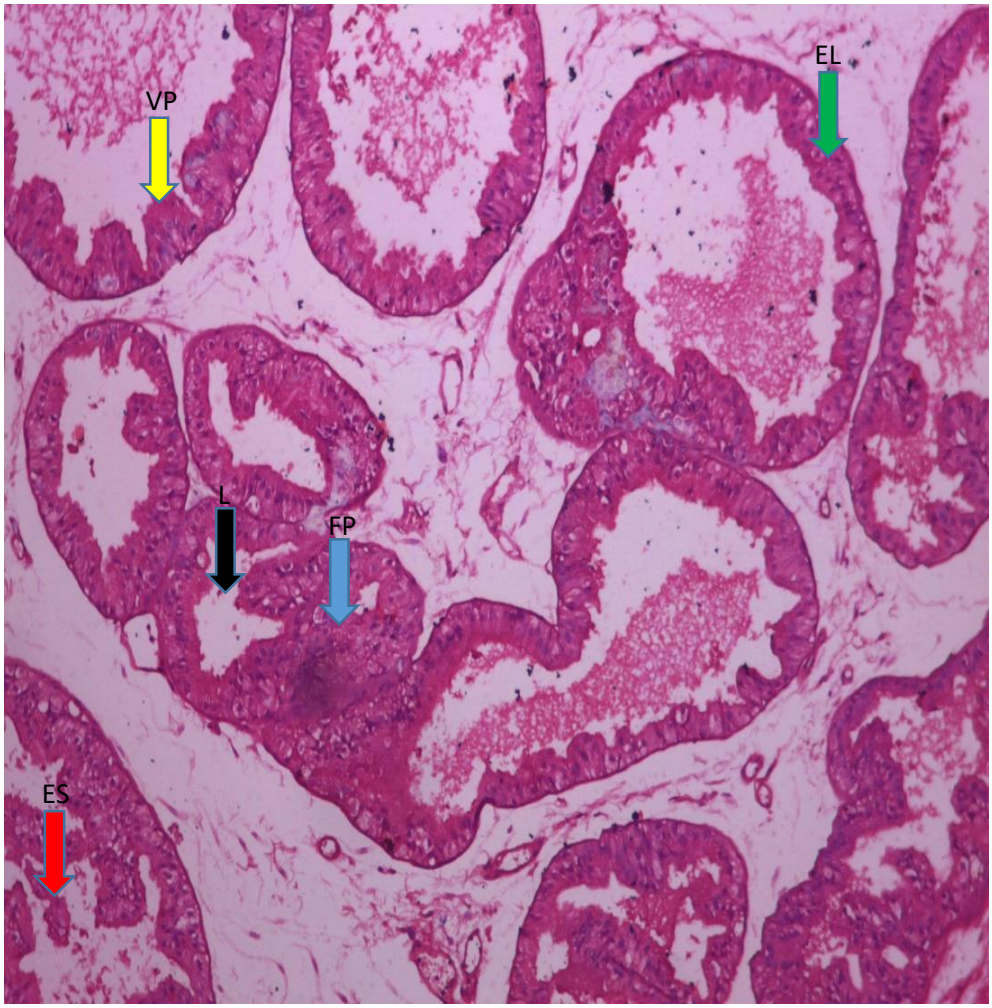

Plate 4.5: Histological section of the prostate from the group treated with 10 ml/kg bw onion juice. Histological section showed single prostatic glands with epithelial sloughing (ES). There was also marked increase in thickness of the epithelium (EL), and villous projections into the lumen (VP), resulting in the narrowing of the lumen (L) (H & E stain x400).

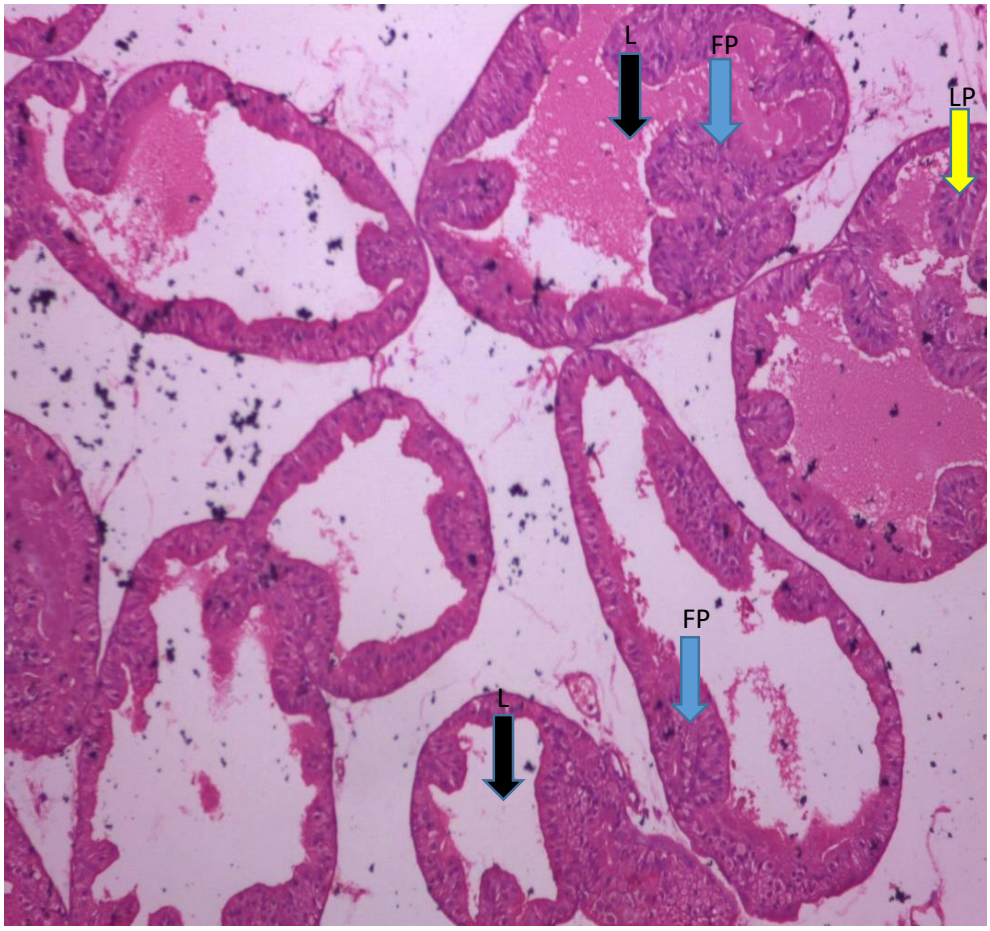

Plate 4.6: Histological section of the prostate from the group treated with 10 ml/kg bw 1:1 soursop and onion juice. Histological section showed single prostatic glands. Luminal projections (LP) and focal proliferation (FP) were present resulting in narrowing lumen (L) (H & E stain x400).

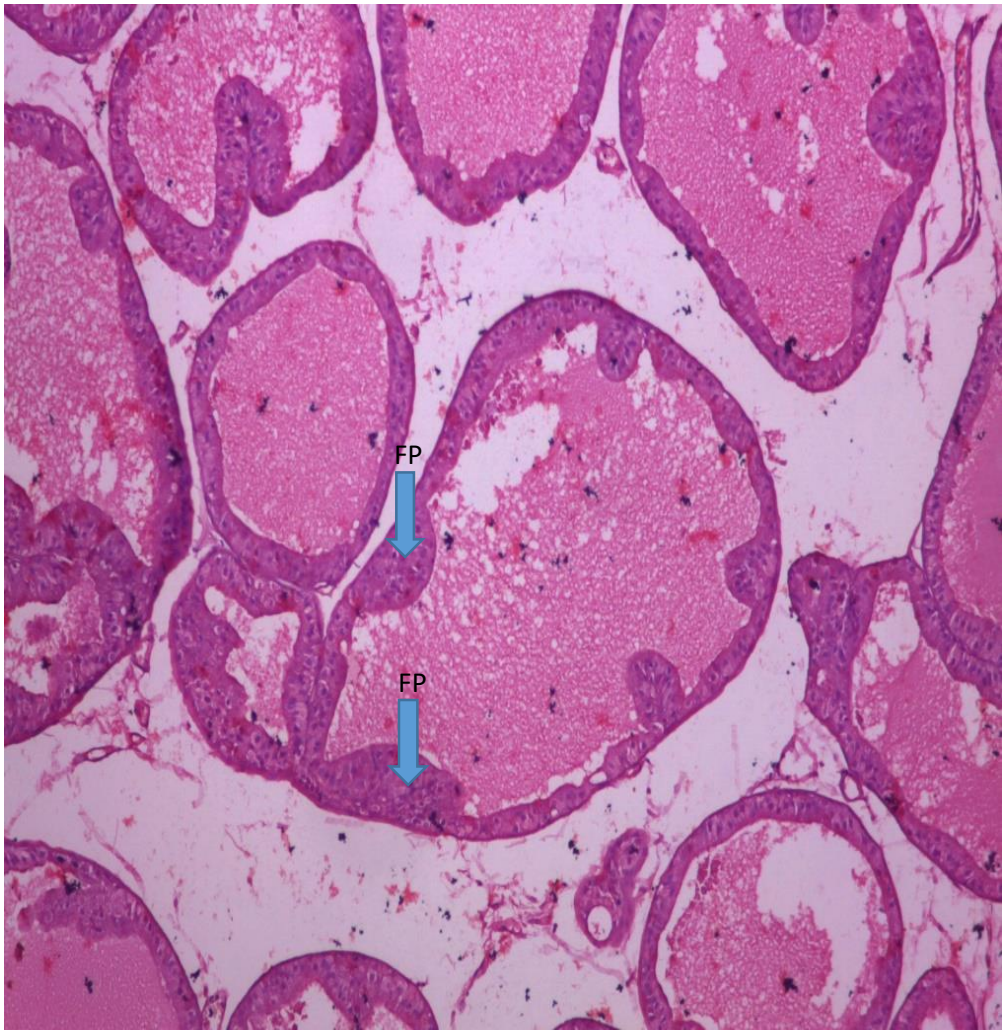

Plate 4.7: Histological section of the prostate from the group treated with 20 ml/kg bw soursop juice. Histological section showed single prostatic glands with focal proliferation (FP). There was mild increase in epithelial lining and few luminal projections (H & E stain x400).

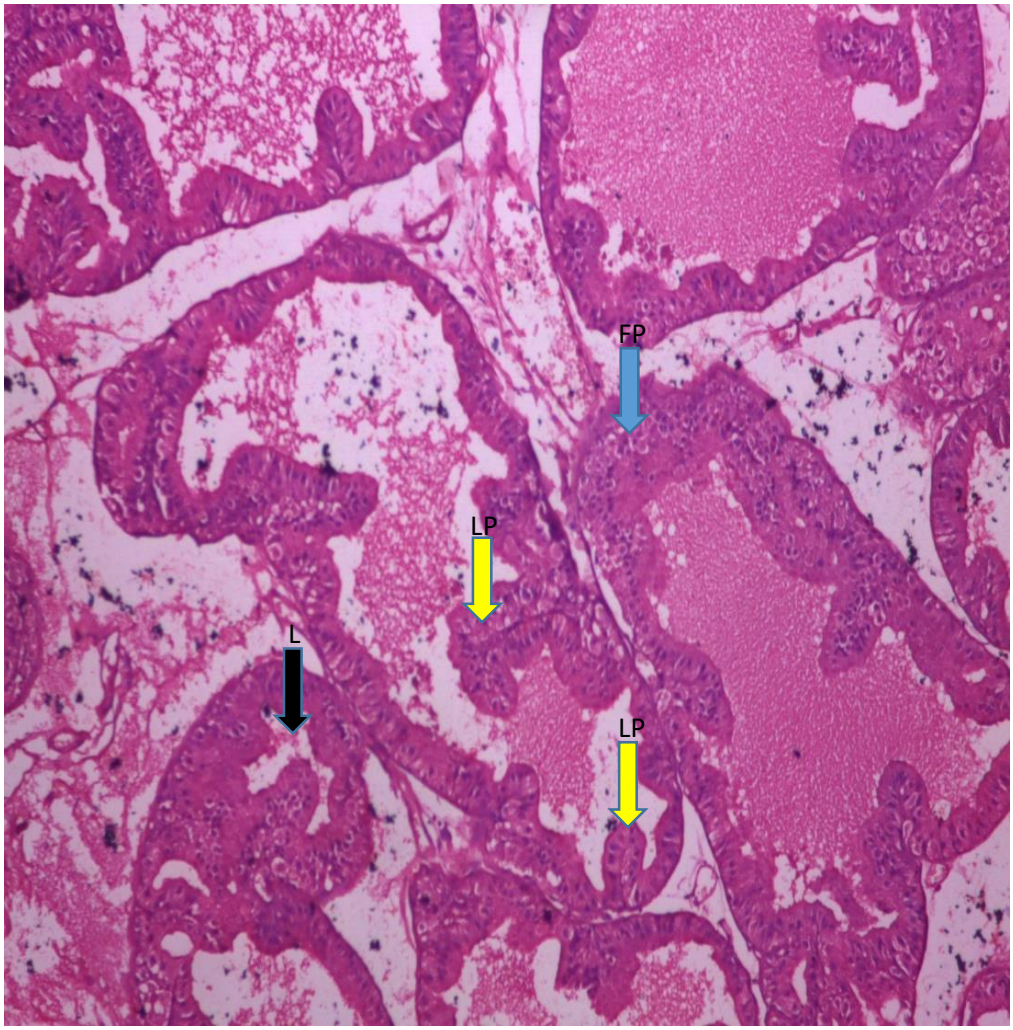

Plate 4.8: Histological section of the prostate from the group treated with 20 ml/kg bw onion juice. Histological section showed single prostatic glands with epithelial luminal projections (LP) narrowing the lumen (L). There was also increased epithelium thickness and focal proliferation (FP) (H & E stain x400).

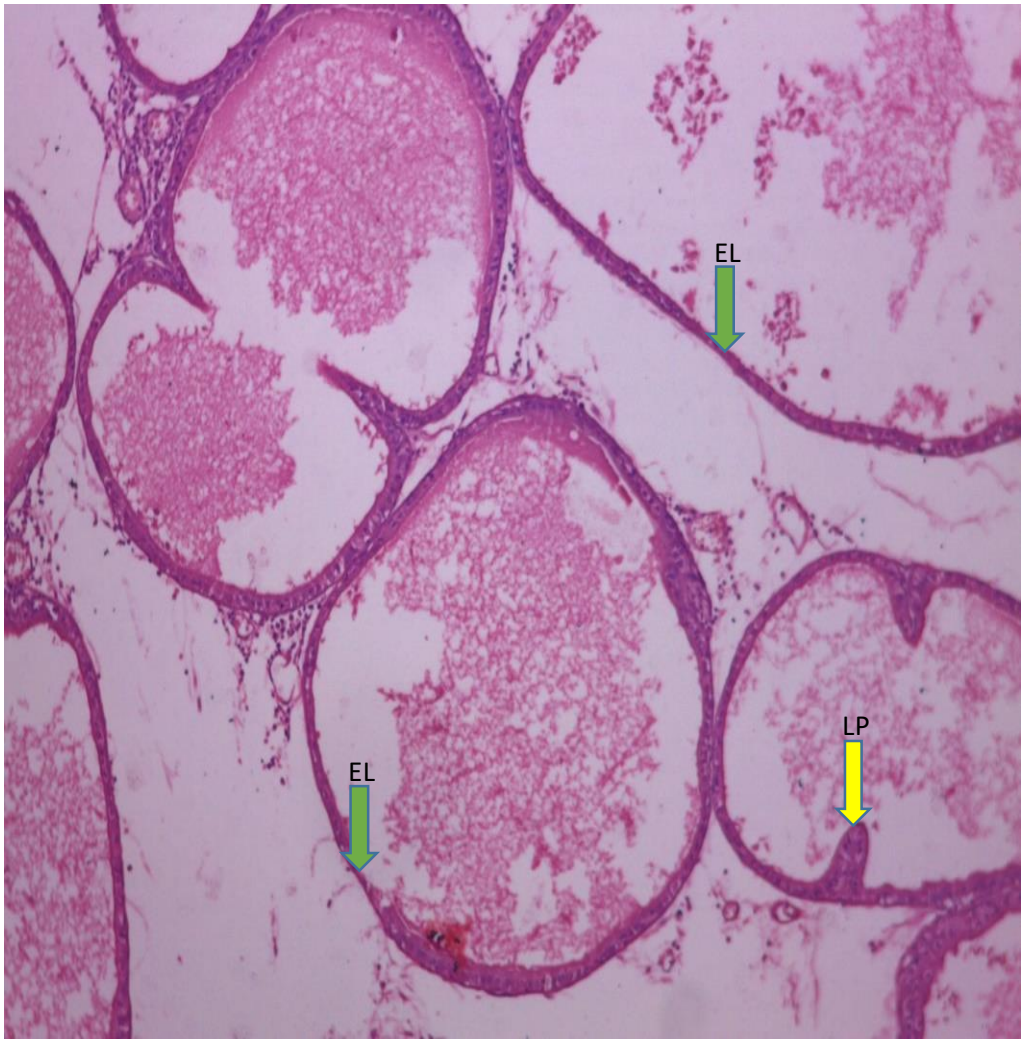

Plate 4.9: Histological section of the prostate from the group treated with 20 ml/kg bw 1:1 soursop and onion juice. Histological section showed prostatic glands singly arranged with thin epithelial lining (EL) infiltrated by lymphocytes. Very few luminal projections (LP) were present (H & E stain x400).

4.1.18 Effect of onion bulb and soursop pulp juices on serum levels of DHT, testosterone, prolactin and PSA of albino rats with testosterone and oestradiol-induced BPH

Table 4.17 presents the effect of *Allium cepa* bulb and *Annona muricata* pulp juices on serum levels of dihydrotestoterone, testosterone, prolactin and PSA of albino rats with testosterone and oestradiol-induced BPH. The induced untreated group (disease control) had significantly (p<0.05) higher levels of DHT, testosterone, prolactin and PSA when compared to the normal control. Treatment with the standard drug and extracts produced a significant reduction (p<0.05) in these parameters, except for the PSA concentrations of the standard control and the low dose groups. This reduction is associated with increase in concentration (Table 4.17).

Table 4.17: Effect of *Allium cepa* bulb and *Annona muricata* pulp juices on serum levels of dihydrotestoterone, testosterone, prolactin and PSA of albino rats with testosterone and oestradiol-induced BPH

Group	Dihydrotestosterone (ng/ml)	Testosterone (ng/ml)	Prolactin (ng/ml)	PSA (ng/ml)
NC	8.02 ± 0.20^{bc}	2.30 ± 0.40^{a}	1.00 ± 0.30^{a}	0.20 ± 0.00^{ab}
DC	10.50 ± 0.60^{d}	10.20 ± 0.70^{f}	1.80 ± 0.10^{b}	0.40 ± 0.10^{c}
SC	8.70 ± 0.50^{c}	6.40 ± 0.20^{b}	1.00 ± 0.10^{a}	0.40 ± 0.20^{c}
10 ml/kg AM	9.10 ± 0.10^{c}	9.80 ± 0.50^{f}	0.90 ± 0.30^{a}	0.30 ± 0.10^{bc}
10 ml/kg AC	8.10 ± 0.80^{bc}	7.30 ± 0.60^{c}	1.10 ± 0.10^{a}	0.30 ± 0.00^{bc}
10 ml/kg AMAC	7.10 ± 1.20^{b}	8.30 ± 0.20^{de}	1.00 ± 0.30^{a}	0.40 ± 0.10^{c}
20 ml/kg AM	5.30 ± 0.80^{a}	9.60 ± 0.10^{f}	0.90 ± 0.00^{a}	0.20 ± 0.10^{ab}
20 ml/kg AC	7.20 ± 0.30^{b}	8.70 ± 0.00^{e}	1.00 ± 0.01^{a}	0.10 ± 0.00^{a}
20 ml/kg AMAC	8.40 ± 0.20^{c}	7.90 ± 0.10^{cd}	0.80 ± 0.20^{a}	0.20 ± 0.10^{ab}

Values are represented as mean ± standard deviation of triplicate determinations. Values on the same column bearing different superscript letters are significantly different (p<0.05).

4.1.19 Effect of onion bulb and soursop pulp juices on prostatic concentration of IL-8, TNF-α, and TNF-β (lymphotoxin-α) of albino rats with testosterone and oestradiol-induced BPH

Table 4.18 presents the effect of *Allium cepa* bulb and *Annona muricata* pulp juices on prostatic concentration of IL-8, TNF-α, and TNF-β (lymphotoxin-α) of albino rats with testosterone and oestradiol-induced BPH. There was significant (p<0.05) reduction in the levels of IL-8, TNF-α, and TNF-β (lymphotoxin-α) in the test groups compared with the disease control (Table 4.18). The extracts dose-dependently reduced TNF-α concentration, and were significantly lower than the standard control except the high dose combined juice. The individual samples dose-dependently decreased lymphotoxin-α, and all were significantly lower than the standard control. For IL-8 concentration, the individual juices dose-dependently caused a decrease when compared with the standard control. The values for soursop treated groups were significantly lower, while those of the onion juice and combined juice were significantly (p<0.05) higher and statistically similar, respectively.

Table 4.18: Effect of *Allium cepa* bulb and *Annona muricata* pulp juices on prostatic concentration of IL-8, TNF-α, and TNF-β (lymphotoxin-α) of albino rats with testosterone and oestradiol-induced BPH

Group	IL-8 (pg/ml)	TNF–α (pg/ml)	TNF-β (Lymphotoxin- α) (pg/ml)
NC	13.3 ± 0.30^{d}	28.0 ± 4.00^{bc}	13.80 ± 0.70^{a}
DC	54.4 ± 0.40^{g}	59.0 ± 3.00^{f}	88.24 ± 1.01^{g}
SC	17.0 ± 2.00^{e}	50.0 ± 8.00^{e}	67.95 ± 1.12^{d}
10 ml/kg AM	7.4 ± 0.00^{b}	24.0 ± 1.00^{b}	62.69 ± 3.01^{c}
10 ml/kg AC	3.8 ± 0.20^{a}	35.0 ± 3.00^{d}	81.84 ± 5.54^{f}
10 ml/kg AMAC	4.0 ± 0.20^{a}	23.0 ± 3.00^{b}	70.93 ± 0.83^{d}
20 ml/kg AM	7.2 ± 0.20^{b}	14.0 ± 0.00^{a}	38.33 ± 1.72^{b}
20 ml/kg AC	9.6 ± 0.20^{c}	16.0 ± 1.00^{a}	76.76 ± 0.70^{e}
20 ml/kg AMAC	32.4 ± 0.00^{f}	31.0 ± 3.00^{cd}	70.93 ± 1.71^{d}

Values are represented as mean ± standard deviation of triplicate determinations. Values on the same column bearing different superscript letters are significantly different (p<0.05).

4.1.20 Effect of onion bulb and soursop pulp juices on PCNA and Caspase-3 activity scores of albino rats with testosterone and oestradiol-induced BPH

Table 4.19 shows the effect of *Allium cepa* bulb and *Annona muricata* pulp juices on PCNA and Caspase-3 activity scores of albino rats with testosterone and oestradiol-induced BPH. The PCNA scores decreased significantly for the soursop juice at low and high doses, compared with the standard control, while the values increased with increase in a dose dependent manner for the groups treated with onion juice and combined juice; although the value for the low dose onion juice compared well with the standard. The caspase-3 activity scores for groups treated with soursop juice and the combined juice reduced in a dose-dependent manner. In comparing with the standard control, the value obtained from the soursop low dose group was significantly (p<0.05) higher, while that of the high dose was not significantly (p>0.05) different. The rest were significantly (p<0.05) lower than the standard control (Table 4.19).

Table 4.19: Effect of *Allium cepa* bulb and *Annona muricata* pulp juices on PCNA and Caspase-3 activity scores of albino rats with testosterone and oestradiol-induced BPH

Group	PCNA (%)	Caspase-3 (%)
NC	7.0 ± 1.00^{a}	32.0 ± 1.73^{b}
DC	70.0 ± 6.56^{f}	5.0 ± 1.00^{a}
SC	36.0 ± 2.65^{c}	80.0 ± 4.58^{f}
10 ml/kg AM	25.0 ± 1.00^{b}	85.0 ± 2.00^{g}
10 ml/kg AC	40.0 ± 3.61^{cd}	62.0 ± 2.65^{c}
10 ml/kg AMAC	56.0 ± 3.51^{e}	67.0 ± 3.06^{d}
20 ml/kg AM	25.0 ± 1.73^{b}	80.0 ± 1.00^{f}
20 ml/kg AC	43.0 ± 3.51^{d}	75.0 ± 4.00^{e}
20 ml/kg AMAC	60.0 ± 2.65^{e}	61.0 ± 1.53^{c}

Values are represented as mean ± standard deviation of triplicate determinations. Values on the same column bearing different superscript letters are significantly different (p<0.05).

4.1.21 Effect of onion bulb and soursop pulp juices on prostatic 5 α-reductase enzyme activity of albino rats with testosterone and oestradiol-induced BPH

Table 4.20 shows the effect of *Allium cepa* bulb and *Annona muricata* pulp juices on prostatic 5 α-reductase enzyme activity of albino rats with testosterone and oestradiol-induced BPH. The 5-α reductase enzyme activity in the prostates of the test groups were reduced, although, the reductions were not significantly different ($p>0.05$) when compared with the standard control (Table 4.20).

Table 4.20 Effect of *Allium cepa* bulb and *Annona muricata* pulp juices on prostatic 5 α-reductase enzyme activity of albino rats with testosterone and oestradiol-induced BPH

Group	5 α-reductase enzyme activity (ng/ml)
NC	0.50 ± 0.10^{a}
DC	2.50 ± 0.20^{e}
SC	1.70 ± 0.10^{cd}
10 ml/kg AM	1.50 ± 0.00^{c}
10 ml/kg AC	1.80 ± 0.10^{cd}
10 ml/kg AMAC	1.20 ± 0.20^{b}
20 ml/kg AM	2.00 ± 0.30^{d}
20 ml/kg AC	1.70 ± 0.00^{cd}
20 ml/kg AMAC	1.70 ± 0.20^{cd}

Values are represented as mean ± standard deviation of triplicate determinations. Values on the same column bearing different superscript letters are significantly different ($p<0.05$).

4.1.22 Inhibitory activity onion bulb and soursop pulp juices on 5-α reductase enzyme

Table 4.21 presents the inhibitory activity of *Allium cepa* bulb and *Annona muricata* pulp juices on 5-α reductase enzyme. The standard drug (Finasteride) showed the highest inhibitory activities on 5 α-reductase enzyme as evident in the testosterone concentration being the highest. This is followed by the soursop juice. Higher testosterone concentration means higher inhibition of 5 α-reductase activity (Table 4.21).

Table 4.21: Inhibitory activity of *Allium cepa* bulb and *Annona muricata* pulp juices on 5-α reductase enzyme

S/N	Sample	Testosterone concentration (ng/ml)
1	Onion juice	9.23 ± 0.06^{a}
2	Soursop juice	11.63 ± 0.15^{b}
3	Combined juice (1:1 soursop and onion juices)	9.90 ± 0.27^{a}
4	Finasteride	13.50 ± 0.10^{c}

Values are represented as mean ± standard deviation of triplicate determinations. Values on the same column bearing different superscript letters are significantly different ($p<0.05$).

4.1.23 Presentation of the packaged *Annona muricata* (soursop) pulp juice

Plate 4.10: The packaged *Annona muricata* (soursop) fruit pulp juice.

Plate 4.10 shows the picture of the packaged *Annona muricata* (soursop) fruit pulp juice. Following the results obtained, the most effective test extract, being soursop pulp juice, was packaged in bottles and labelled properly.

4.1.24 Sensory evaluation result of the soursop juice

Table 4.22 presents the results of sensory evaluation of the soursop pulp juice. The result of sensory evaluation showed that the colour, taste and aroma of the soursop juice were rated very good, while the mouth feel and overall acceptability were rated good (Table 4.22).

Table 4.22: Results of sensory evaluation of the soursop pulp juice

Attributes	Score	Rating
Colour	4.39 ± 0.49	Very good
Taste	4.11 ± 0.31	Very good
Mouth feel	3.42 ± 0.69	Good
Aroma	4.42 ± 0.50	Very good
Overall acceptability	3.89 ± 0.32	Good

Values are represented as mean ± standard deviation of twenty-eight responses.

4.1.25 Results of microbiological screening

Table 4.23 shows the results of microbiological screening of soursop pulp juice. The results obtained showed that microbial growth only occurred on the NA, TSI and PDA media. The most probable organism on NA was observed to be *Bacillus subtilis*, while *Saccharomyces cerevisiae* was the most probable organism on TSI and PDA. The microbial counts were 4.0×10^4 and 1.0×10^5 cfu/ml for the *Bacillus subtilis* and *Saccharomyces cerevisiae*, respectively (Table 4.23).

Table 4.23: Results of microbiological screening of soursop pulp juice

S/N	Culture media	Cultural characteristics	Most probable organism	Microbial count (cfu/ml)
1	NA	Creamy spherical and flat colonies with wrinkled surface. Diameter of 4 mm.	*B. subtilis*	4.0×10^4
2	MCA	No growth	-	-
3	SSA	No growth	-	-
4	TSI	Creamy spherical, glossy and raised colonies. Diameter of 10 mm.	*S. cerevisiae*	1.0×10^5
5	PDA	Creamy, glistening, spherical, and raised colonies. Diameter of 5 to 10 mm.	*S. cerevisiae*	1.0×10^5

4.2 Discussion

Benign prostatic hyperplasia (BPH) is a progressive disease condition in aging men. It is accompanied by lower urinary tract symptoms (LUTS) (Emberton et al., 2008), and affects the quality of life in patients. Its pathophysiology remains unknown but aging is a major factor that has been reported to be involved in the development of BPH. Other factors include, metabolic syndrome, hormonal changes, and inflammation (Berger et al., 2005).

Although, BPH is uncommon before age 40, but by 50 years of age, about 50 % of men begin to experience symptoms related to BPH, and this number rises to 80 % by the age of 80 (Bharti, 2017). There is no cure yet for BPH, chemotherapeutic agents, mainly alpha blockers and 5-α reductase inhibitors, have been in use and quite helpful, but not without side effects (Kim et al., 2018; Unal et al., 2020).

The cost of conventional therapy and their possible side effects are the major reasons for shift towards complementary and alternative medicine (CAM) for diverse ailments. Medicinal plants provide a major source of CAM for various ailments, as they are perceived to be cheaper, safer and with lesser side effects (Calixto, 2000; Thompson et al., 2003).

The presence of phytochemicals in medicinal plants are responsible for their therapeutic potentials, resulting in their utilization in the management and treatment of certain ailments. Soursop and onion are rich in phytochemical compounds responsible for the array of activities they exert that is beneficial to human health. These activities, proven by studies, include antimicrobial, antiprotozoan, anti-inflammatory, antioxidant, and anticancer effects (Matsuura, 2001; Lanzotti, 2006; Gyesi et al., 2019). These potentials, therefore, present onion and soursop as possible plants to source for phytotherapeutic agents for the treatment and management of BPH.

Table 4.1 presents the results of LD_{50} of the juice samples of soursop fruit pulp and onion bulb. The LD_{50} is the dose of a substance capable of causing death in 50% (half) of the total population of animals exposed to the substance. Both juices of soursop pulp and onion bulb were safe, even at the highest concentration of 5,000 mg/kg bw. This results agreed with the reports of other researchers. Okeke-Nwolisa, Enweani-Nwokelo, Unekwe and Egbuonu (2023) reported that the LD_{50} of hydro-ethanolic extract of ripe soursop fruit pulp is above 5,000 mg/kg bw. While, Wahdaningsih, Untari and Robiyanto (2019) reported LD_{50} of ethanolic extract of Dayak onion leaves to be greater than 5,000 mg/kg bw.

Tables 4.2 to 4.11 present the *in vitro* antioxidant properties of juice samples of soursop fruit pulp and onion bulb. The parameters analyzed were FRAP, nitric oxide scavenging property, superoxide, hydrogen peroxide, DPPH, ABTS, and hydroxyl radical scavenging activities, total phenol content, total antioxidant capacity, and total flavonoids content.

Antioxidants, also referred to as free radical scavengers, constitute a defence mechanism of the body, protecting it from the negative effects of reactive oxygen species (ROS) on the cells (Jayachitra & Krithiga, 2010). A variety of factors result in the production ROS, which include stress, respiration, lifestyle factors, toxins. There are endogenous antioxidants synthesized by the body, while the body also relies on exogenous antioxidants supplied through diets (Valko et al., 2007), especially from plant sources (fruits, vegetables and grains) (Bouayed & Bohn, 2010).

Antioxidant activity of a plant depends on the presence of certain biologically active compounds, especially polyphenols, carotenoids, and vitamin E and C (Baiano & Del Nobile, 2016). Reducing power is frequently used to assess the antioxidant activity of plant polyphenols, typically linked with the presence of reductants, which exert antioxidant action by donation of hydrogen atom to break the free radical chains. Radical scavenging activities occurring within tissues are very important in preventing the deleterious effects of free radicals in the development of different diseases, including cancer.

From the results of this study, it was observed that both extracts possessed all the *in vitro* antioxidant properties assessed in varying capabilities/degrees.

Soursop juice showed nitric oxide scavenging capacity, total phenol content, total antioxidant capacity, and hydrogen peroxide scavenging activity. At the highest concentration of soursop juice, the total antioxidant capacity was better than the reference compound.

Onions, however, showed higher ferric reducing antioxidant property, higher total flavonoids content in all the samples that also compares well with the reference. Onion juice showed very strong ABTS scavenging activity/potential even better than the reference in all the samples. Onion juice also showed higher hydroxyl radical scavenging activity. Athough, the hydroxyl radical scavenging activity for both juices were observed to decrease in a dose-dependent manner.

Onion juice showed higher superoxide and DPPH scavenging activities, while soursop juice showed higher values at the least concentration.

These findings agreed with the earlier works of Škerget et al. (2009), Singh et al. (2009), Santas et al. (2010), Gupta, Pandey, Shah, Yadav and Seth (2011), Agu and Okolie (2017), Fredotović et al. (2017) who reported that plants are natural sources of exogenous antioxidants. Gupta et al. (2011) attributed antioxidant properties of plants to phytoconstituents, such as, polyphenols, flavonoids, tannins, alkaloids, annonaceous acetogenins, and essential oils.

The results of the effect of *Allium cepa* bulb and *Annona muricata* pulp juiceson the body weight and percentage body weight change of the albino rats are presented in Tables 4.12 and 4.13. Brai, Odetola and Agomo (2007) stated that body weight measurements are used as indicators of overall health. The results of this study indicated that at the low dose of the extracts (soursop juice, onion juice, and combined), there was steady increase in body weights of the animal models as was also observed in the normal and standard controls. Hence, at low dose, body weights of the albino rats were maintained.

The percentage body weight change obtained for the low dose groups were positive for all the weeks, further consolidating that the body weights were maintained. The average percentage body weight change in these groups compared to standard control group, which was treated with the standard drug (Finasteride), indicated that the extracts at low dose ensured body weight maintenance in the same manner as the standard drug.

Table 4.14 presents results of the effect of juice samples of *Annona muricata* pulp and *Allium cepa* bulb on prostate weight (PW), prostate index (PI), relative prostate weight (RPW) and percentage prostate increase inhibition (PPII) of the albino rats. The reduction in PW in all the test groups signalled response to treatment, with the lesser values observed in the low dose groups indicating that the extracts were more effective at reducing prostate weight at low doses. This means that increasing the dosage of the extracts did not cause further reduction in the PW. For PI and RPW, only values from groups treated with low dose soursop juice, and high doses of soursop juice and combined juice elicited significant reduction. This reduction means that soursop juice improved these parameters. The low dose groups inhibited prostate increase better, with the highest inhibition observed in group treated with onion juice at low dose, followed by the group treated with low dose of soursop juice. Figures from the high dose groups also indicated that increase in dosage inhibited prostate increase poorly.

Changes in indices associated with prostate weight have been used by previous researchers to monitor effects of different substances on BPH development and/or progression (Arruzazabala et al., 2006; Ammar et al., 2015). The results of this study agrees with the findings of Ogbu

and Aloke (2020), which stated that actogenin-rich fraction of *Annona muricata* leaves extract reduced PW and RPW in treated rats.

Tables 4.15 and 4.16 shows the effect of juice samples of *Annona muricata* pulp and *Allium cepa* bulb on selected markers of oxidative stress of albino rats with induced BPH. The parameters assessed were superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione S-transferase (GST), Catalase (CAT), Malondialdehyde (MDA), reduced glutathione (GSH), total protein and protein carbonyl (PCO) groups activities /concentrations. Available information in literature associated the development of BPH with oxidative stress (Minciullo et al., 2015). Oxidative stress is capable of causing damage to cells, tissues, as well as organs, and impairing functions of important biomolecules (Durackova, 2010; Udensi & Tchounwou, 2016). Antioxidants, enzymes and compounds, protect cellular structures by interfering with the actions of ROS by specific mechanisms (Sachdev, Ansari, Ansari, Fujita & Hasanuzzaman, 2021). An imbalance in oxidative stress and antioxidant system within cellular environments is a key factor in the development of BPH. Previous studies have always reported reduced antioxidant levels in the prostates and sera of BPH models (Ammar et al., 2015; Park et al., 2016).

The results of this present study agrees with the reports of these previous investigations as evident in the reduction of *in vivo* antioxidant systems in the disease control, which is the BPH model. In this study, the test samples induced a reduction in MDA, total protein and PCO groups concentrations, while increasing GSH concentration and SOD, GPx, GST and CAT activities.

Malondialdehyde is a lipid peroxidation marker which increases in concentration with increase in free radicals. Except for the group treated with low dose of onion juice, the extracts at their different doses reduced lipid peroxidation in a manner similar to either the standard or normal control.

Protein carbonyl groups levels is a biomarker of and accepted as gold standard for measuring protein oxidation (Luddi et al., 2020). The reduction in protein carbonyl groups concentration in the test groups indicated that the test samples reduced protein oxidation. The soursop juice caused reduction in this parameter in a dose-dependent manner, while the reverse was the case with the onion juice and combinations.

Superoxide dismutase (SOD), GPx, GST, CAT and GSH are endogenous antioxidant enzymes and antioxidants. The SOD catalyzes superoxide radicals dismutation to yield hydrogen

peroxide and molecular oxygen; GPx reduces hydrogen peroxide and lipid peroxides to non-toxic forms. The GST mainly catalyses the conjugation of reduced glutathione to electrophilic and hydrophilic compounds from xenobiotic biotransformation, CAT catalyzes the breakdown of hydrogen peroxide to oxygen and water, while GSH plays a major role in the detoxification of various electrophilic compounds.

In this study, there were significant ($p<0.05$) improvements in the oxidative status of the rats in the test groups when compared with the disease control, except for GSH level in the group treated with the low dose of onion juice, which was statistically similar to the disease control. All these observations in these oxidative stress markers in the respective test groups reiterated the ability of the extracts to alleviate oxidative stress. The impaired oxidative status observed in the disease control in this study agrees with the study by Aryaal et al. (2007) that stated that oxidative stress is associated with BPH. Zabaiou, Mabed, Lobaccaro and Lahouel (2015) also reported that the development of BPH is accompanied by impaired oxidative status indicated by increased MDA levels, depletion of GSH and reduced activities of antioxidant enzymes.

Histopathological examination of organs and tissues is also an important tool for monitoring effects of different substances on the development and progression of disease conditions; BPH is not exempted (Arruzazabala et al., 2006; Ammar et al., 2015). In this study, prostate tissue from the experimental animals were examined to identify effects of *Allium cepa* bulb and *Annona muricata* pulp juice extracts on BPH development and progression. The photomicrographs are presented as plates 4.1 to 4.9.

The histological architecture of the prostate from the test groups showed that the extracts ameliorated BPH in varying capacities when compared with the disease control (Plate 4.2), with Plate 4.6 (histology of prostate from the group treated with soursop juice at low dose) showing stronger indication of alleviating BPH.

Table 4.17 presents the results of the effect of juice extracts of *Allium cepa* bulb and *Annona muricata* pulp on serum DHT, testosterone, prolactin and PSA levels of BPH induced albino rats.

DHT and testosterone are androgens which play prime roles in the development of the human male reproductive system internal organs, and BPH (Andriole et al., 2004; Lucia & Lambert, 2008; Miller & Tarter, 2009). Testosterone is converted to DHT catalyzed by 5 α-reductase enzyme. DHT is the more active androgen that stimulates the growth of the epithelial and stromal cells of the prostate, due to its high affinity (2- to 5-fold when compared to testosterone)

to the androgen receptors (Mizokami et al., 2009; Nicholson & Ricke, 2011, Da Silva & De Souza, 2019; Vickman et al., 2020).

In this study, the observed reduction in DHT and testosterone levels in all test groups and the standard control meant that these extracts have therapeutic effects. While the individual extracts decreased DHT levels with increase in concentration, soursop juice and the combination decreased testosterone levels in a dose-dependent manner.

It was observed that the prolactin levels were significantly reduced in both the test groups and standard control when compared with the disease control. The PSA levels were also reduced in the test groups.

PSA is a glycoprotein that is predominantly produced in the prostate gland. BPH is an age-related condition, and its prevalence increases with increase in age. With increasing age in males, prostate volume increases. This increasing prostate volume with age results in increasing PSA levels (Putra et al., 2016). Although PSA values greater than 4.0 ng/ml is considered abnormal in men above 60 years of age, yearly increase in PSA levels greater than 0.35 ng/ml is a cause for concern and requires further testing, irrespective of a patient's previous normal PSA levels (Pavlovich, 2023). Prolactin has also been implicated in development of BPH, as it targets the prostate through interactions with the prolactin receptors expressed in the prostate (La Vignera, Condorelli, Russo, Morgia & Calogero, 2016). The results from this study is in agreement with the work of van Coppenolle et al. (2001) who stated that chronic hyperprolactinemia has also been implicated in inflammation and hypertrophy of the lateral rat prostate. Cooper (2019) opined that high oestrogen and prolactin levels also increase DHT levels and stimulate growth of prostate cells.

Inflammation is frequently present in BPH, which could lead to tissue injury. As a self-defense mechanism, inflammatory cells secrete cytokines that can promote angiogenesis and synthesis of local growth factor in the tissues (Lucia & Lambert, 2008). According to previous studies, proinflammatory cytokines like IL-8 and TNF-α are potent growth factors for increase in prostatic epithelial and stromal cells in BPH models (Penna et al., 2009; Yang, Yuan, Xiong, Yin & Ruan, 2014). Consequently, many studies have reported the use of agents with properties of anti-inflammation in BPH (Chung, An, Cheon, Kwon & Lee, 2015; Kim et al., 2016). Lymphotoxin-α (LTα, previously known as TNF-β) is a member of the TNF family and closest homolog to TNFα. It is primarily synthesized by activated T and B lymphocytes (Naoum et al.,

2006), and has been implicated in autoimmunity and inflammation (Calmon-Hamaty, Combe, Hahne & More, 2011).

In this study, it was observed that there were significant ($p<0.05$) reductions in IL-8, TNF-α and LT-α levels in the prostate of the test groups when compared with the disease control. This observation suggests that anti-inflammatory property can be a part of the mechanisms employed by the extracts in the attenuation of BPH. For IL-8, only the soursop juice caused reduction with increase in dosage, while for TNF-α and LT-α, both the soursop and onion juice extracts also caused reduction with increase in dosage.

PCNA is a DNA polymerase δ auxiliary protein particularly expressed in proliferating cell nuclei. The PCNA expression in cell has been recognized as the cell cycle's G1/S phase marker, which is related to the pathogenesis of BPH (Bantis et al., 2004; Kim et al., 2016). The results from this study, as presented in Table 4.19, showed a significant decrease in PCNA score in the test groups compared to the disease control. The increase in PCNA expression observed in the diseases control agrees with the study of Raafat et al. (2022) that observed a marked increase in PCNA expression in untreated BPH group. Zhang, Niu and Huang (2021) also reported that there is a connection between PCNA expression and tissue proliferation

Caspase-3 is a crucial enzyme in apoptosis involved in the pathogenesis of BPH; an apoptotic biomarker too (Atawia, Tadros, Khalifa, Mosli & Abdel-Naim, 2013; Chung et al., 2015). This study showed, also in Table 4.18, an increased caspase-3 expression in finasteride treated group (standard control) as consistent with previous studies (Bozec et al., 2005). There was also an increase in caspase-3 expression in the test groups, meaning that the extracts' effect on BPH development and progression could involve antiproliferative and proapoptotic activity. The significantly low caspase-3 expression score observed in the disease control agrees with work of Zhang, Zhang, Zhang, Na & Guo (2006). Their report stated that the development of BPH may be associated with active proliferation of stromal cell and epithelial growth due to decrease in glandular apoptosis.

The 5-α reductase (5αR) enzyme catalyzes the conversion of testosterone to DHT. In the prostate, there are two isoenzymes of 5αR that are abundant; 5αR1 and 5αR2. Increase in expression of 5αR2 indicates BPH (Cavallo & Kaplan, 2018). In this study, as shown in Table 4.20, it was observed that there was a significant increase in 5αR2 enzyme on BPH induction, which was reduced in the test groups and the finasteride treated groups. The manner in which the test groups, except for the group treated with high dose soursop juice, reduced 5αR

concentration in the prostate were not significantly ($p>0.05$) different from the standard control (Finasteride treated group). This suggested that the extracts also employ the reduction of expression of 5αR2 in their bid to ameliorate BPH.

As previously mentioned, DHT is more potent than testosterone in BPH development. *In vitro* inhibition study was carried out to determine the effects of the extracts on 5 α-reductase-mediated conversion of testosterone to DHT. The ability of samples to inhibit the conversion of testosterone to DHT suggested that 5-α reductase enzyme activity was inhibited with more testosterone remaining unchanged in the reaction mixture (Nahata, Agrawal & Dixit, 2017). The results, as presented in Table 4.21, showed that the soursop juice had more inhibitory action on the enzyme in relation to Finasteride, when compared with other test samples.

The results of this research led to soursop juice being chosen for packaging. The soursop pulp juice, being the most effective test extract, was packaged in bottles and labelled properly (Plate 4. 10). Hence, the soursop juice was subjected to further preliminary assessment to determine safety. These assessments included sensory evaluation, and microbiological screening.

Sensory evaluation measures, analyzes, and people's reactions to products based on their senses (Stone, 2018). The result of sensory evaluation (Table 4.22) showed rating ranging from good to very good. This showed that the soursop juice was acceptable. This observation agreed with the earlier study of Onyechi, Ibeanu, Okechukwu and Nsofor (2018). They reported a high rating of taste and overall acceptability of fresh soursop pulp over sweetened and unsweetened freeze-dried and oven-dried soursop pulp.

Microbiological screening of food is important in determining the safety of food. It provides information on the quality of food, sanitary and storage conditions of the food. Additionally, it exposes the types of microorganisms in food, the quality of raw materials used and evaluates the effectiveness of methods of food preservation used (Karki, 2020). The results of this study (Table 4.23) showed the presence of *B. subtilis* and *S. cerevisea* in the soursop juice. *B. subtilis* is a gram positive and aerobic soil bacterium (Earl, Losick & Kolter, 2008), catalase positive and rod shaped. It is considered non-pathogenic to humans (Tsonis et al., 2018). It is also found in association with plants (Stork et al., 2021), and in the gut of mammals (Kimelman & Shemesh, 2019) and safely consumed by humans (Brutscher, Borgmeir, Garvey & Spears, 2022). Some *B. subtillis* strains are have been studied for use by humans as probiotics, such as CU1 (Lefevre et al., 2017), M6 (Ji et al., 2022), and MB40 (Piewngam et al., 2023).

S. cerevisea is a unicellular fungus that finds application in many industries for fermentation (Parapouli, Vasileiadis, Afendra & Hatziloukas, 2020). It is regarded as a human-friendly organism (Morard et al., 2023). It is found on fruits, leaves, nectars, insects, and the environment, and are the sources of *S. cerevisea* contamination. Just like *B. subtilis*, some *S. cerevisea* strains have been beneficial to human health as supplements and probiotics (Sen & Mansell, 2020). The microbial counts of the microorganisms in the soursop juice were within acceptable limits. Onuoha et al. (2018) reported that the acceptable limit for microbial contamination safe for human consumption is 10^6 cfu/ml as stated by the International Commission on Microbiological Specifications for Food (ICMSF). Hence, the presence of *B. subtilis* and *S. cerevisea* in the soursop juice is not necessarily a cause for concern.

Furthermore, care should be taken when handling/consuming fresh unpasteurized juices, such as the soursop juice in this study, to avoid food poisoning and to obtain optimal benefits. As such, hygiene, temperature, and time, should be put into consideration. The best time to consume fresh unpasteurized juices is immediately after etraction. But when it is to be kept, it is advised that the two-hour rule should be taken seriously. The two-hour rule recommends that fresh unpasteurized juice left at room temperature for up to two hours should be deemed unsafe afterwards (United States Food and Drug Administration, 2017; Center for Disease Control and Prevention, 2023; United States Department of Agriculture, 2023). Kuddumukasa, Imathiu, Mathara, and Nakavuma (2017) also suggested that when fresh unpasteurized juice is hygienically prepared, it can be safe for consumption for 48 hours when held at refrigeration temperature (4 ^{o}C).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

From the findings of this study, it can be concluded that the juice extracts of *Allium cepa* (onion) bulb and *Annona muricata* (soursop) pulp possessed *in vitro* antioxidant potentials. This is with regards to the ferric reducing antioxidant property, nitric oxide scavenging property, superoxide scavenging activity, total phenol content, total antioxidant capacity, total flavonoids content, hydroxyl radical scavenging ability, hydrogen peroxide scavenging property, DPPH and ABTS scavenging activities. Hence, soursop pulp and onion bulb juice are sources of exogenous antioxidants.

This research further probed into the effect of the juice extracts of *Allium cepa* bulb and *Annona muricata* fruit pulp on testosterone and oestradiol-induced BPH in albino rats. The findings showed that both extracts and their combinations exerted ameliorative effects in varying degrees, at low and high doses. These effects include maintenance of body weight, reduction of prostate weight, improving *in vivo* antioxidant status, hormonal and PSA levels. Other effects are anti-inflammatory and antiproliferative potentials, as indicated by the levels/expression of cytokines, cytotoxic and pro-apoptotic factors/proteins, and biomarkers. Considering the values obtained from this research emanating from the test groups, it can be stated that soursop juice exhibited/produced better ameliorative effects, more especially, at low dose. The photomicrographs showing histopathological architecture of the prostate from these groups buttresses this statement.

Sensory evaluation and microbiological screening of the soursop juice showed that it is acceptable to humans and safe for human consumption.

5.2 Recommendations for further studies

It is therefore recommended that;

1. Soursop is a very beneficial fruit and its consumption should be encouraged, especially by older men.
2. Soursop juice should be processed and packaged in a way that it will have longer shelf-life, such that it can be available all year round, as it is a very seasonal fruit.

3. Further research should be conducted to demonstrate the effects of these plant extracts and their combinations on other factors/proteins implicated in the pathogenesis of BPH. Examples include IL-6, IL-2, IL-5, insulin-like growth factors, caspase-9, oestrogen.
4. Studies should be carried out using these extracts to establish their effects on other vital organs and health indices in association with BPH treatment.
5. *In vivo* studies should be conducted, using animal models, with the incorporation of the individual extracts, especially soursop fruit pulp, with the standard drug to identify mode of interaction (synergistic or antagonistic) of both.

5.3 Contributions to knowledge

Onion and soursop juices ameliorated BPH. These juices employed a number of mechanisms in ameliorating BPH, which included;

1. Improved anti-oxidative status by elevating SOD, CAT, GPx, and GST activities, increased glutathione (reduced) concentration, reduced lipid peroxidation, and reduced the concentrations of total protein and protein carbonyl groups.
2. Reduced concentrations of DHT, testosterone, prolactin and PSA.
3. Lowered prostatic levels of IL-8, TNF-alpha and Lymphotoxin-alpha.
4. Suppressed PCNA expression, while also elevating Caspase-3 activity.
5. Inhibited activity of 5-α reductase enzyme.

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APPENDIX A

ETHICAL APPROVAL

FEDERAL UNIVERSITY OF TECHNOLOGY, OWERRI
SCHOOL OF BIOLOGICAL SCIENCES
DEPARTMENT OF BIOCHEMISTRY

VICE-CHANCELLOR
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Date: 27/10/2021

DEPARTMENTAL ETHICAL COMMITTEE

Mrs. Hope Amarachi Ihejieto
Department of Biochemistry
Postgraduate School
Federal University of Technology
Owerri.

Dear Mrs. Hope Amarachi Ihejieto

RE: REQUEST FOR ETHICAL APPROVAL

We hereby respond to your application seeking approval for the use of rats for your research on the effect of onion bulb and soursop pulp juices on benign prostrate hyperplasia in rats.

Your application has been considered by members and approved. You are to ensure that the way you handle the animals/rats meet the requirements of the National Institutes of Health, NIH (2011); by treating them humanely.

Congratulation!

Prof. R.N. Nwaogukpe
(Chairman)

APPENDIX B

REAGENT PREPARATION

Sodium citrate buffer

Weigh out 2.94 g of trisodium citrate (dehydrate). Dissolve in 900 ml of deionized, distilled water. Adjust the pH to 6.0 with 1.0 N HCl. Add 0.5 ml of tween-20. Mix and bring up the volume to 1 l with water. This can be stored at room temperature for 3 months or longer at 4 ^{o}C.

Vanillin reagent

vanillin sulfuric acid reagent was prepared by dissolving 1 g of vanillin added with 2 ml of sulfuric acid in 100 ml of 95% ethanol.

Sodium phosphate buffer

Place 800 mL of distilled water in a suitable glassware. Add 20.214 g of sodium phosphate dibasic heptahydrate to the solution. Add 3.394 g of sodium phosphate monobasic monohydrate to the solution. Adjust solution to final desired pH using HCl or NaOH. Add distilled water until the volume is 1 L.

Potassium phosphate buffer

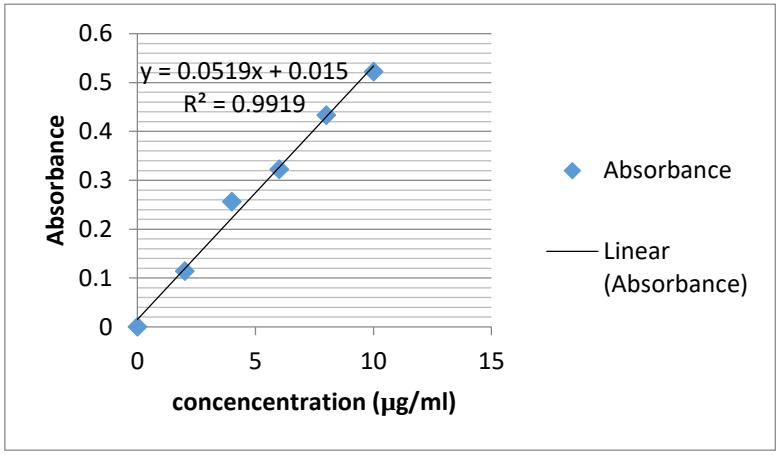
Place 800 mL of distilled water in a suitable glassware. Add 95 g of potassium phosphate monobasic to the solution. Add 52.5 g of Potassium phosphate dibasic to the solution. Adjust the pH to 6.5. Add distilled water until the volume is 1 L. Filter, sterilize and store at room temperature.

Phosphate buffered saline 1x, pH 7.4

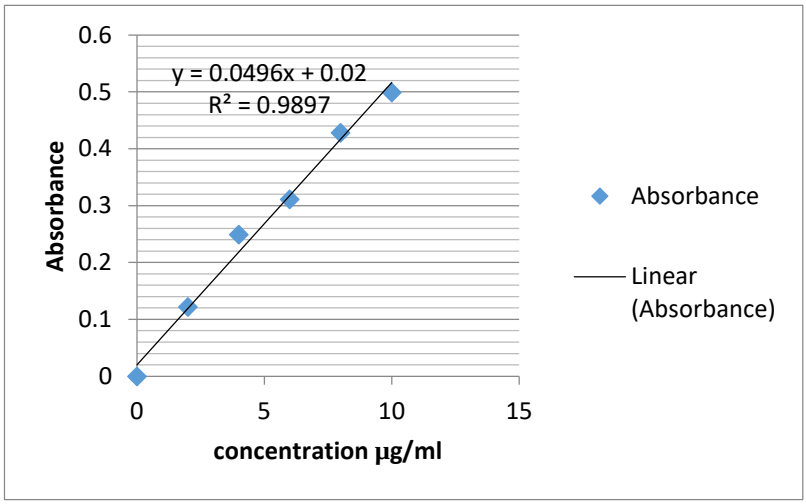
Place 800 mL of distilled water in a suitable glassware. Add 8 g of sodium chloride to the solution. Add 0.2 g of potassium chloride to the solution. Add 1.44 g of Sodium Phosphate Dibasic to the solution. Add 0.245 g of potassium phosphate monobasic to the solution. Adjust solution to desired pH (typically pH ≈ 7.4). Add distilled water until the volume is 1 L.

APPENDIX C

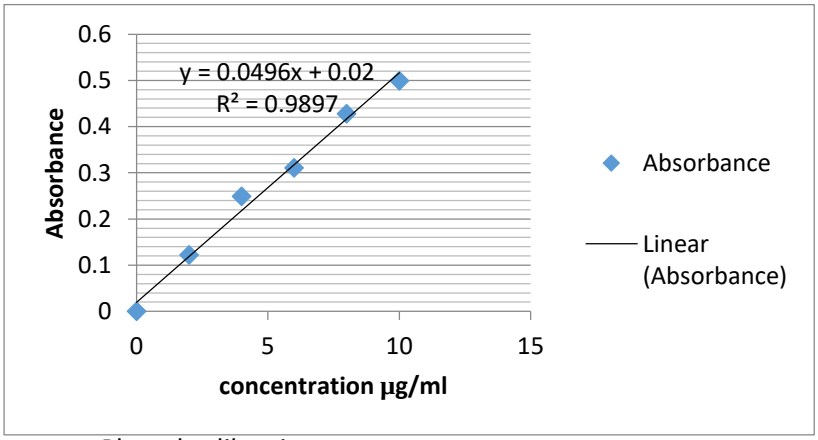
STANDARD CURVES



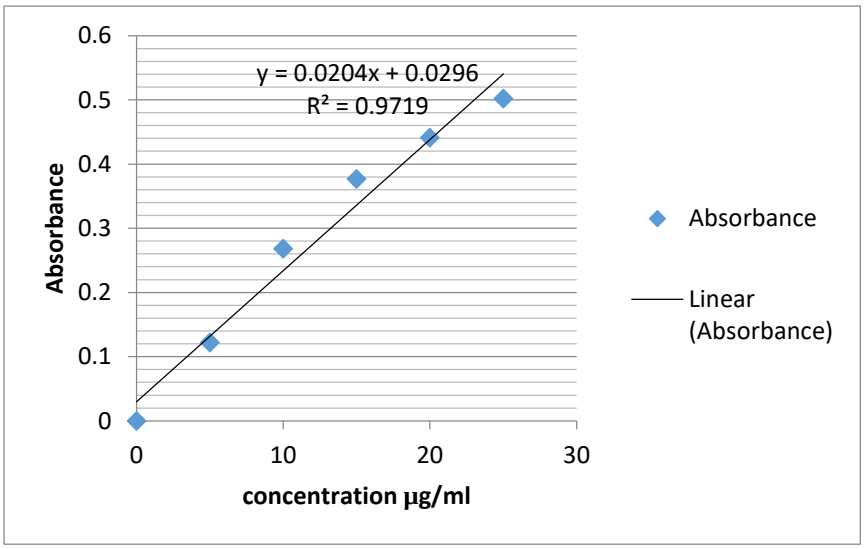
FRAP Calibration curve



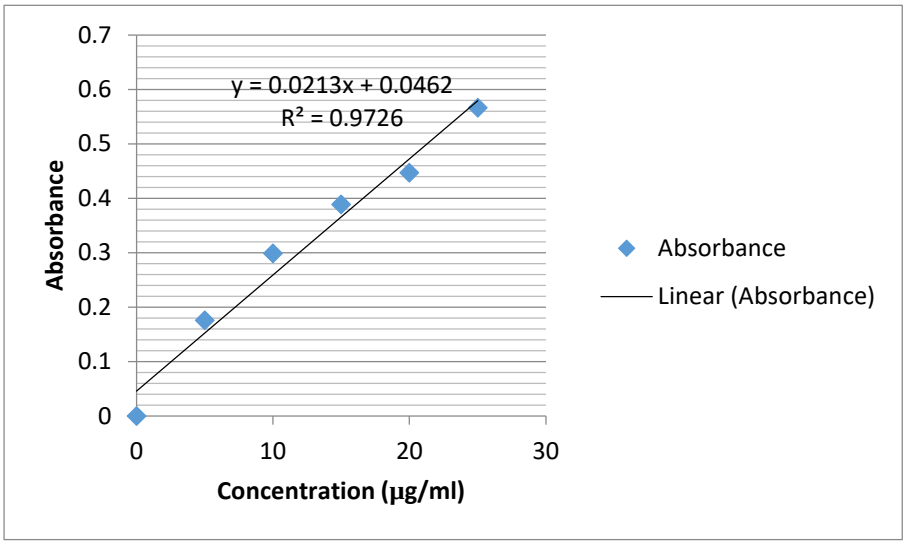
Nitric oxide calibration curve



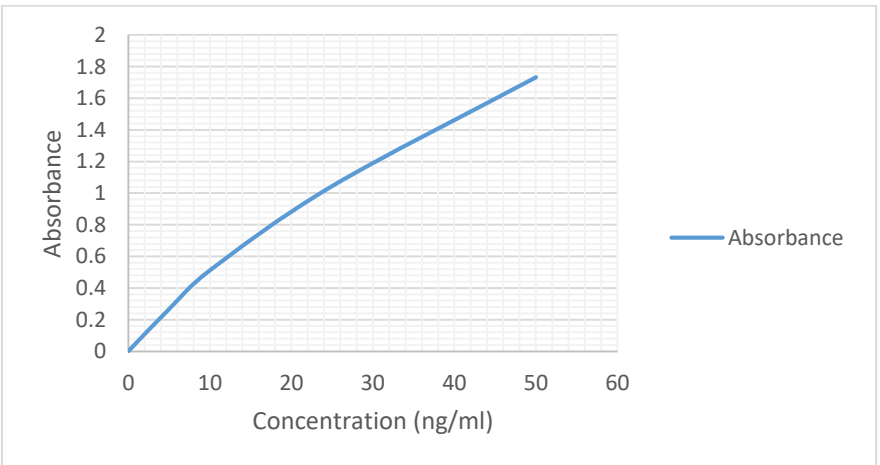
Phenol calibration curve



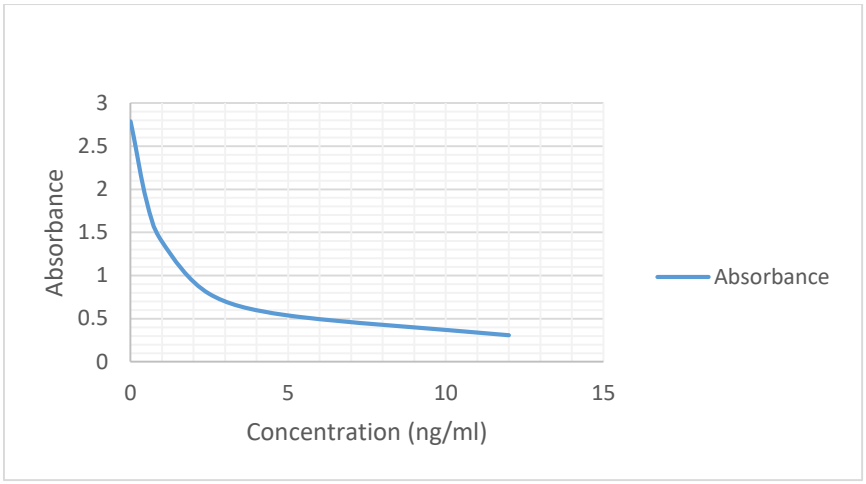
TAC calibration curve



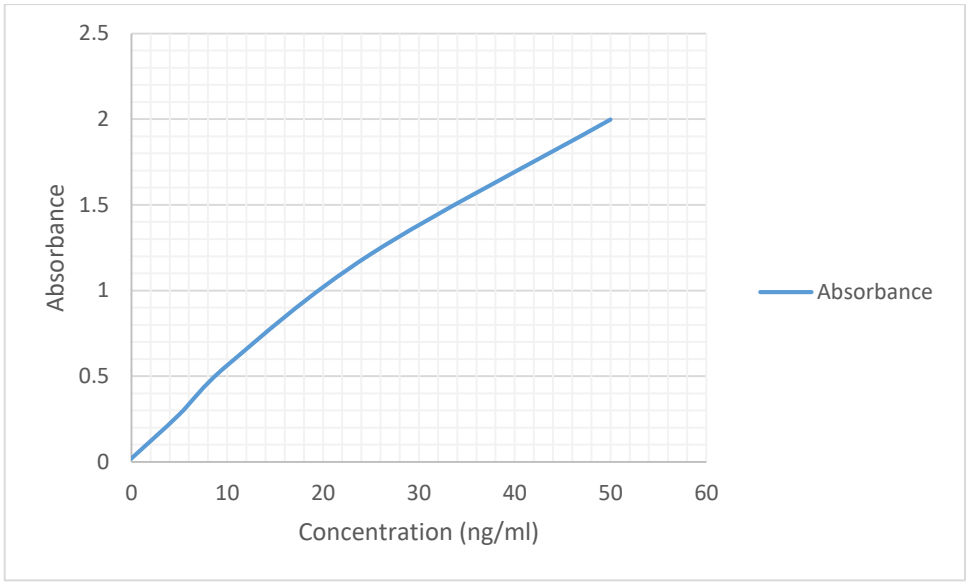
Total flavonoids calibration curve



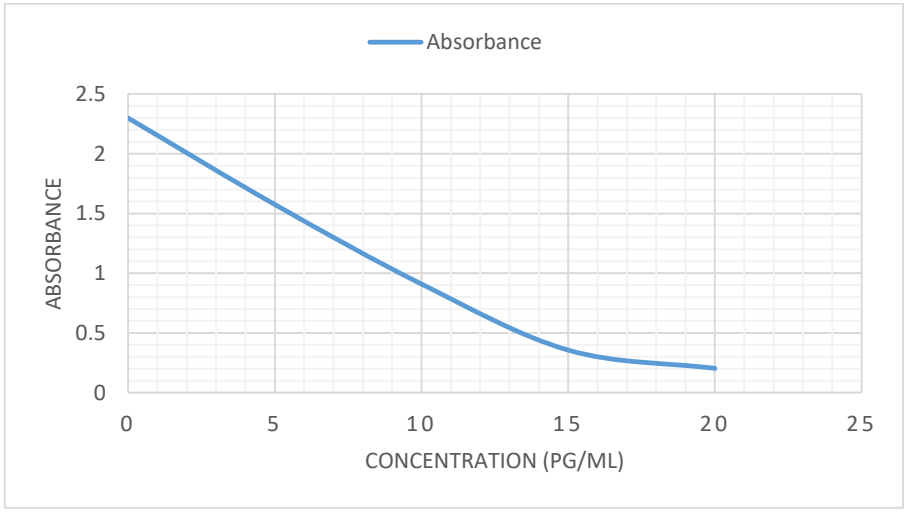
Prolactin calibration curve



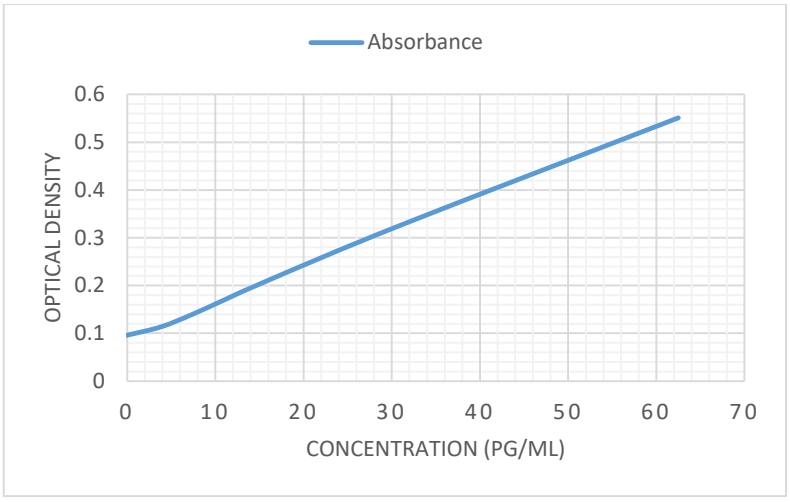
Testoterone calibration curve



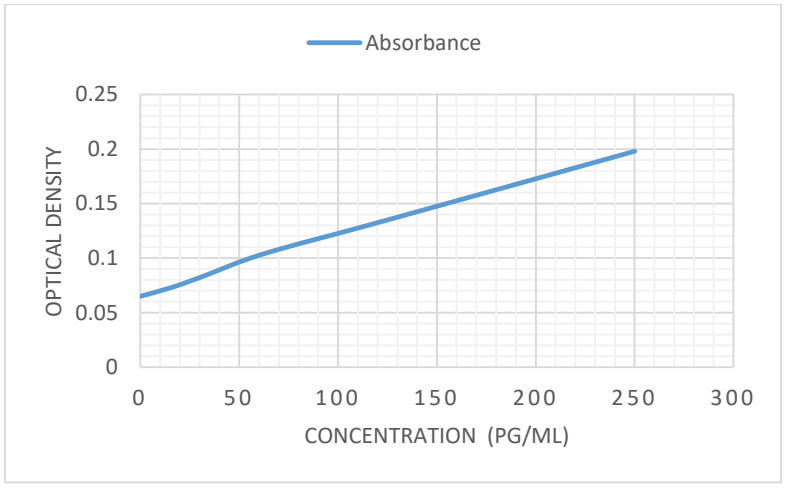
PSA calibration curve



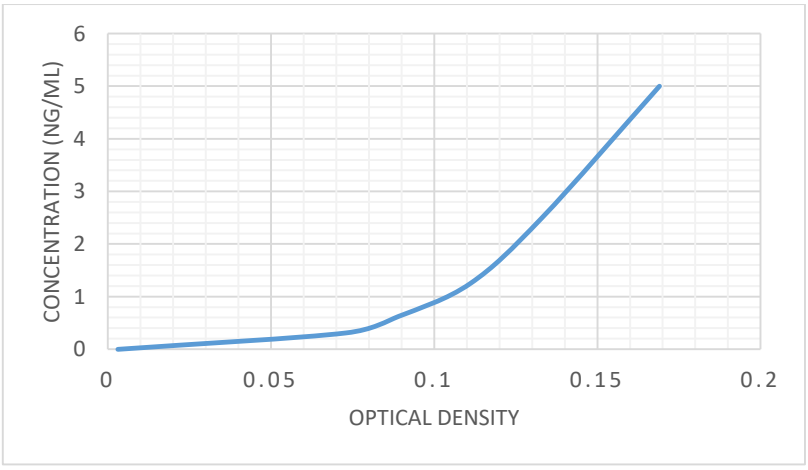
DHT calibration curve



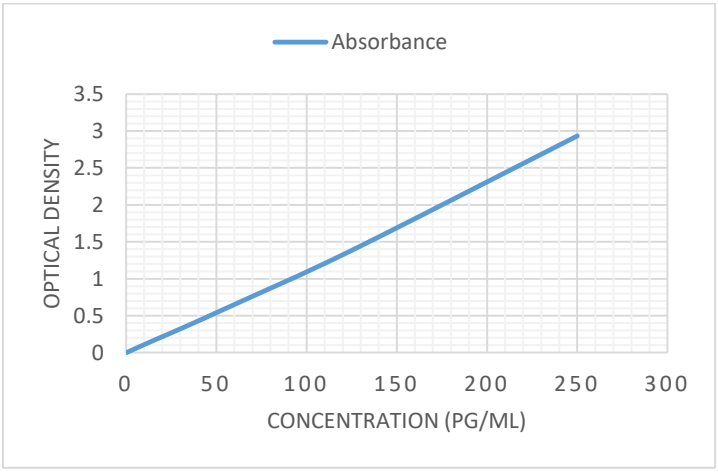
IL-8 calibration curve



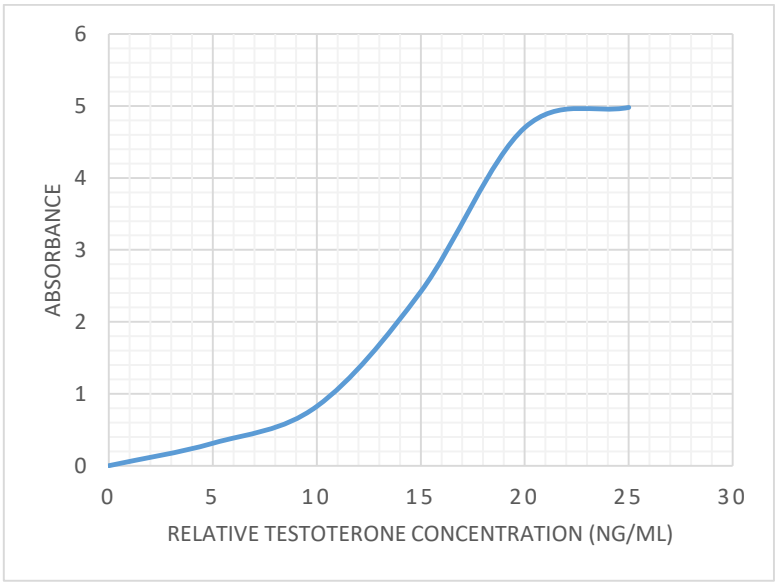
TNF-alpha calibration curve



5-alpha reductase calibration curve



TNF-beta calibration curve



In vitro 5-alpha reductase inhibition calibration curve

APPENDIX D

QUESTIONNAIRE FOR SENSORY EVALUATION

Taster's code:..... **Date:.....**

You have been presented with a sample of juice. Drink and evaluate the colour, taste, texture, aroma and overall acceptability. Tick (√) the appropriate rating column that matches your response in the table below:

1. Response of taster:

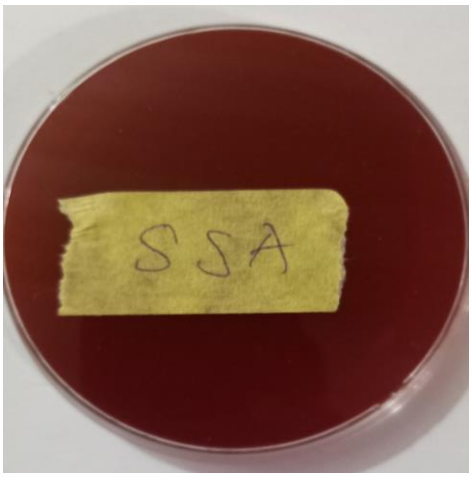
Rating scale	**Attributes**				
	Colour	Taste	Mouth feel	Aroma	Overall acceptability
5. Excellent					
4. Very good					
3. Good					
2. Satisfactory					
1.Poor					
0.Unacceptable					

2. Additional comments:.....

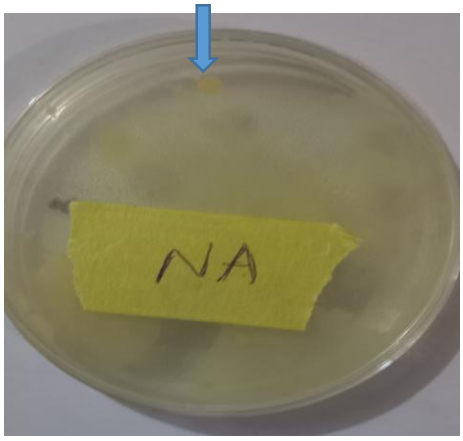
APPENDIX E

THE MICROBIAL PLATES

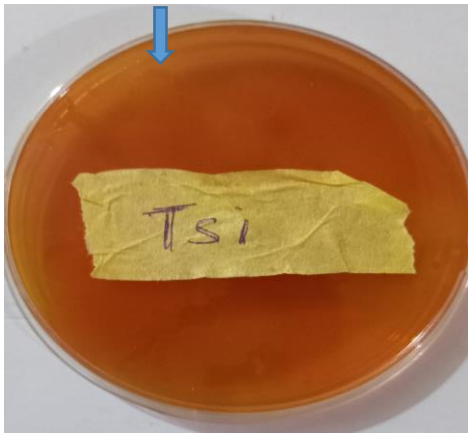
Picture of MCA plate showing no growth



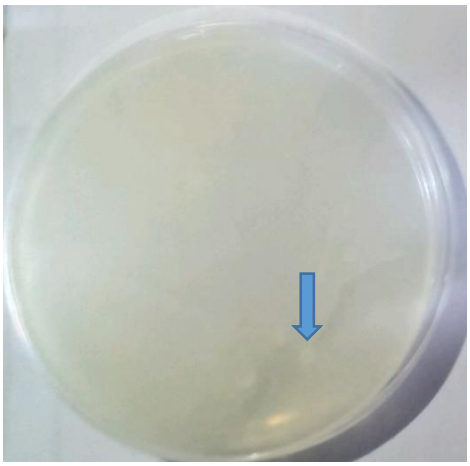
Picture of MCA plate showing no growth



Picture of NA plate showing growth



Picture of TSI plate showing growth



Picture of PDA plate showing growth

APPENDIX F

STATISTICAL ANALYSIS

```
GET
  FILE='C:\Users\USER\Documents\invitro Untitled1.sav'.
DATASET NAME DataSet1 WINDOW=FRONT.
ONEWAY frapsampleA frapsampleB NitricoxidesampleA NitricoxidesampleB SuperoxidesampleA
    SuperoxidesampleB HydrogenperoxidesampleA HydrogenperoxidesampleB
HydroxylradicalsampleA
    HydroxylradicalsampleB TotalphenolcontentsampleA TotalphenolcontentsampleB
    TotalantioxidantcapacitysampleA TotalantioxidantcapacitysampleB TotalfavonoidsampleA
    TotalflavonoidsampleB DPPHsampleA DPPHsampleB ABTSsampleA ABTSsampleB BY group
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=DUNCAN ALPHA(0.05).
```

Oneway

Post Hoc Tests

Homogeneous Subsets

frapsampleA

Duncan[a]

group	N	Subset for alpha = 0.05			
		1	2	3	4
100	3	1.6665			
200	3		1.7963		
300	3			2.2550	
100mg/ml	3				3.5875
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

frapsampleB

Duncan[a]

group	N	Subset for alpha = 0.05	
		1	2
100	3	2.6278	
200	3	2.7060	
300	3	2.7545	
100mg/ml	3		3.5875
Sig.		.058	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

NitricoxidesampleA

Duncan[a]

group	N	Subset for alpha = 0.05	
		1	2
100	3	2.3875	
200	3	2.5205	
300	3	2.5293	
100mg/ml	3		3.4385
Sig.		.438	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

NitricoxidesampleB

Duncan[a]

group	N	Subset for alpha = 0.05	
		1	2
100	3	2.2860	
200	3	2.3200	
300	3	2.4600	
100mg/ml	3		3.4385
Sig.		.223	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

SuperoxidesampleA

Duncan[a]

group	N	Subset for alpha = 0.05			
		1	2	3	4
100	3	54.6890			
200	3		57.6563		
300	3			61.8317	
100mg/ml	3				70.3125
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

SuperoxidesampleB

Duncan[a]

group	N	Subset for alpha = 0.05			
		1	2	3	4
100	3	52.8543			
200	3		58.1250		
300	3			63.7210	
100mg/ml	3				70.3127
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

HydrogenperoxidesampleA

Duncan[a]

group	N	Subset for alpha = 0.05		
		1	2	3
300	3	47.4410		
200	3	47.5410	47.5410	
100	3		47.7170	
100mg/ml	3			60.7627
Sig.		.263	.067	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

HydrogenperoxidesampleB

Duncan[a]

group	N	Subset for alpha = 0.05			
		1	2	3	4

300	3	46.2120			
200	3		46.5380		
100	3			47.2400	
100mg/ml	3				60.6957
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

HydroxylradicalsampleA

Duncan[a]

		Subset for alpha = 0.05			
group	N	1	2	3	4
300	3	14.5450			
200	3		18.3337		
100	3			30.4547	
100mg/ml	3				80.4540
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

HydroxylradicalsampleB

Duncan[a]

		Subset for alpha = 0.05			
group	N	1	2	3	4
300	3	41.1575			
200	3		42.4435		

100	3			65.9397	
100mg/ml	3				80.4540
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

TotalphenolcontentsampleA

Duncan[a]

		Subset for alpha = 0.05			
group	N	1	2	3	4
100	3	2.9900			
200	3		4.8677		
300	3			6.3067	
100mg/ml	3				9.6737
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

TotalphenolcontentsampleB

Duncan[a]

		Subset for alpha = 0.05			
group	N	1	2	3	4
100	3	1.9900			
200	3		2.6530		
300	3			3.5207	
100mg/ml	3				9.6737

Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

TotalantioxidantcapacitysampleA

Duncan[a]

		Subset for alpha = 0.05			
group	N	1	2	3	4
100	3	3.9750			
200	3		4.5250		
100mg/ml	3			8.8270	
300	3				13.0917
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

TotalantioxidantcapacitysampleB

Duncan[a]

		Subset for alpha = 0.05			
group	N	1	2	3	4
100	3	2.6000			
200	3		3.8750		
300	3			8.2367	
100mg/ml	3				8.8270
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

TotalfavonoidsampleA

Duncan[a]

group	N	Subset for alpha = 0.05	
		1	2
300	3	58.1667	
200	3	58.2140	
100	3	58.3570	
100mg/ml	3		59.3337
Sig.		.084	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

TotalflavonoidsampleB

Duncan[a]

group	N	Subset for alpha = 0.05
		1
100	3	58.8337
200	3	58.8810
300	3	59.1903
100mg/ml	3	59.3335
Sig.		.097

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

DPPHsampleA

Duncan[a]

group	N	Subset for alpha = 0.05			
		1	2	3	4
100	3	85.8672			
200	3		87.2119		
300	3			95.2334	
100mg/ml	3				98.2437
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

DPPHsampleB

Duncan[a]

group	N	Subset for alpha = 0.05		
		1	2	3
100	3	85.4138		
200	3		93.0923	
300	3			97.7725
100mg/ml	3			98.2437
Sig.		1.000	1.000	.344

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

ABTSsampleA

Duncan[a]

group	N	Subset for alpha = 0.05			
		1	2	3	4
100	3	55.2854			
200	3		57.7952		
300	3			59.6195	
100mg/ml	3				61.3108
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

ABTSsampleB

Duncan[a]

group	N	Subset for alpha = 0.05			
		1	2	3	4
100mg/ml	3	61.3108			
100	3		67.1247		
200	3			68.1818	
300	3				71.1417
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

```
DATASET NAME DataSet1 WINDOW=FRONT.
ONEWAY INITIAL_WEIGHT WEEK_1 WEEK_2 WEEK_3
WEEK_4 BY GROUP
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=DUNCAN ALPHA(0.05).
```

Oneway

ANOVA

INITIAL_WEIGHT

Duncan[a]

GROUP	N	Subset for alpha = 0.05			
		1	2	3	4
GROUP_5	3	140.2667			
GROUP_4	3	148.4667	148.4667		
GROUP_6	3	151.6800	151.6800	151.6800	
GROUP_3	3	159.3733	159.3733	159.3733	
GROUP_7	3	163.1767	163.1767	163.1767	
GROUP_1	3		177.1333	177.1333	177.1333
GROUP_9	3		177.9533	177.9533	177.9533
GROUP_8	3			179.8033	179.8033
GROUP_2	3				198.5567
Sig.		.131	.059	.071	.149

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

WEEK_1

Duncan[a]

GROUP	N	Subset for alpha = 0.05			
		1	2	3	4
GROUP_5	3	153.7533			
GROUP_4	3	158.1333	158.1333		
GROUP_6	3	159.9767	159.9767	159.9767	
GROUP_3	3	166.5167	166.5167	166.5167	

GROUP_7	3	171.3600	171.3600	171.3600	171.3600
GROUP_9	3		184.2900	184.2900	184.2900
GROUP_8	3			188.8800	188.8800
GROUP_1	3				196.3100
GROUP_2	3				200.0433
Sig.		.231	.081	.055	.057

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

WEEK_2

Duncan[a]

		Subset for alpha = 0.05		
GROUP	N	1	2	3
GROUP_5	3	161.2967		
GROUP_4	3	162.9333		
GROUP_3	3	167.2367		
GROUP_6	3	167.4300		
GROUP_7	3	172.7000	172.7000	
GROUP_9	3	182.5767	182.5767	
GROUP_8	3	185.6533	185.6533	
GROUP_2	3		197.9200	197.9200
GROUP_1	3			218.1533
Sig.		.083	.062	.102

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

WEEK_3

Duncan[a]

		Subset for alpha = 0.05	
GROUP	N	1	2
GROUP_5	3	162.2300	
GROUP_4	3	168.5433	

GROUP_7	3	168.7967	
GROUP_6	3	170.6500	
GROUP_3	3	175.5367	
GROUP_8	3	180.5733	
GROUP_9	3	182.1300	
GROUP_2	3	189.6800	
GROUP_1	3		235.1800
Sig.		.075	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

WEEK_4

Duncan[a]

		Subset for alpha = 0.05	
GROUP	N	1	2
GROUP_5	3	165.4567	
GROUP_7	3	165.8767	
GROUP_6	3	176.0567	
GROUP_4	3	176.4200	
GROUP_3	3	185.4067	
GROUP_9	3	187.8133	
GROUP_8	3	189.6867	
GROUP_2	3	199.8400	
GROUP_1	3		244.6000
Sig.		.085	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

```
DATASET ACTIVATE DataSet3.
ONEWAY prostateweightg finalbodyweightg prostateindexmg relativeprostateweight BY group
  /STATISTICS DESCRIPTIVES
```

/MISSING ANALYSIS

/POSTHOC=DUNCAN ALPHA(0.05).

Oneway

ANOVA

Post Hoc Tests

Homogeneous Subsets

prostateweightg

Duncan[a]

group	N	Subset for alpha = 0.05		
		1	2	3
rat 1,rat 2,rat 3	3	2.1833		
rat 1,rat 2,rat 3	3	2.5300	2.5300	
rat 1,rat 2,rat 3	3	2.7700	2.7700	2.7700
rat 1,rat 2,rat 3	3		2.8500	2.8500
rat 1,rat 2,rat 3	3		2.9467	2.9467
rat 1.rat 2,rat 3	3		2.9767	2.9767
rat 1,rat 2,rat 3	3		3.0000	3.0000
rat1,rat 2,rat 3	3			3.2500
rat 1,rat 2,rat 3	3			3.3133
Sig.		.063	.155	.106

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

finalbodyweightg

Duncan[a]

group	N	Subset for alpha = 0.05

		1	2
rat 1,rat 2,rat 3	3	165.4567	
rat1,rat 2,rat 3	3	165.8767	
rat 1.rat 2,rat 3	3	176.0567	
rat 1,rat 2,rat 3	3	176.4200	
rat 1,rat 2,rat 3	3	185.4067	
rat 1,rat 2,rat 3	3	187.8133	
rat 1,rat 2,rat 3	3	189.6867	
rat 1,rat 2,rat 3	3	199.8400	
rat 1,rat 2,rat 3	3		244.6000
Sig.		.085	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

prostateindexmg

Duncan[a]

		Subset for alpha = 0.05		
group	N	1	2	3
rat 1,rat 2,rat 3	3	.0093		
rat 1,rat 2,rat 3	3		.0137	
rat 1,rat 2,rat 3	3		.0155	
rat 1,rat 2,rat 3	3		.0160	.0160
rat 1,rat 2,rat 3	3		.0162	.0162
rat 1,rat 2,rat 3	3		.0166	.0166
rat 1,rat 2,rat 3	3		.0169	.0169
rat 1.rat 2,rat 3	3		.0170	.0170
rat1,rat 2,rat 3	3			.0196

Sig.		1.000	.096	.068

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

relativeprostateweight

Duncan[a]

group	N	Subset for alpha = 0.05		
		1	2	3
rat 1,rat 2,rat 3	3	9.2555		
rat 1,rat 2,rat 3	3		13.6869	
rat 1,rat 2,rat 3	3		15.4629	
rat 1,rat 2,rat 3	3		15.9987	15.9987
rat 1,rat 2,rat 3	3		16.1778	16.1778
rat 1,rat 2,rat 3	3		16.5802	16.5802
rat 1,rat 2,rat 3	3		16.8652	16.8652
rat 1.rat 2,rat 3	3		17.0191	17.0191
rat1,rat 2,rat 3	3			19.6377
Sig.		1.000	.096	.068

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

```
GET
  FILE='C:\Users\USER\Documents\NEW AMARA.sav 1.sav'.
DATASET NAME DataSet1 WINDOW=FRONT.
GET
  FILE='C:\Users\USER\Documents\NEW AMARA 2.sav'.
DATASET NAME DataSet2 WINDOW=FRONT.
```

```
DATASET ACTIVATE DataSet1.

ONEWAY SUPEROXIDEDISMUTASE GLUTATHIONE CATALASE GPX TOTALPROTEIN
LIPIDPEROXIDATION PROTEINCARBONYL

  GST INTERLEUKIN FIVEALPHAREDUCTASEOPTIMUMCONCENTRATION TNFALPHA TNFBETA DHT
PROLACTIN

  TESTOSTERONE PSA BY GROUP

 /STATISTICS DESCRIPTIVES

 /MISSING ANALYSIS

 /POSTHOC=DUNCAN ALPHA(0.05).
```

Oneway

Post Hoc Tests

Homogeneous Subsets

SUPEROXIDEDISMUTASE

Duncan[a]

GROUP	N	Subset for alpha = 0.05				
		1	2	3	4	5
group 2	3	45.5200				
group 5	3		58.1403			
group 9	3		60.8643			
group 8	3		64.1757			
group 6	3			76.4083		
group 7	3			79.3887		
group 4	3				91.4823	
group 3	3					100.1010
group 1	3					106.3327
Sig.		1.000	.071	.332	1.000	.052

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

GLUTATHIONE

Duncan[a]

GROUP	N	Subset for alpha = 0.05					
		1	2	3	4	5	6
group 2	3	31.5640					
group 5	3		39.7100				
group 4	3			51.4867			
group 8	3			51.6253			
group 6	3			58.1910	58.1910		
group 9	3				61.5617		
group 3	3				62.0827		
group 7	3					69.5920	
group 1	3						79.1030
Sig.		1.000	1.000	.081	.300	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

CATALASE

Duncan[a]

GROUP	N	Subset for alpha = 0.05						
		1	2	3	4	5	6	7
group 2	3	8.3740						
group 7	3	13.6120	13.6120					
group 1	3		16.9380	16.9380				
group 3	3		17.5170	17.5170				
group 4	3			20.2290	20.2290			
group 5	3				25.9500	25.9500		

group 8	3					30.2840	30.2840	
group 9	3						33.8623	33.8623
group 6	3							37.8317
Sig.		.074	.196	.273	.053	.133	.211	.167

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

GPX

Duncan[a]

GROUP	N	Subset for alpha = 0.05				
		1	2	3	4	5
group 2	3	1.3370				
group 5	3		1.8003			
group 8	3			2.1370		
group 7	3			2.2643		
group 6	3				2.6010	
group 3	3				2.6637	
group 9	3				2.7080	
group 4	3				2.8110	
group 1	3					3.4390
Sig.		1.000	1.000	.219	.068	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

TOTALPROTEIN

Duncan[a]

GROUP	N	Subset for alpha = 0.05					
		1	2	3	4	5	6
group 1	3	12.6890					
group 7	3		15.8167				
group 9	3		16.7500	16.7500			
group 3	3		16.8333	16.8333			
group 6	3			17.5933			
group 8	3				19.7933		
group 4	3				20.0467		
group 5	3					25.2100	
group 2	3						33.3300
Sig.		1.000	.122	.195	.675	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

LIPIDPEROXIDATION

Duncan[a]

GROUP	N	Subset for alpha = 0.05			
		1	2	3	4
group 5	3	.9683			
group 6	3		1.2817		
group 7	3		1.3783		
group 3	3		1.3953		
group 4	3			1.6030	
group 9	3			1.6120	
group 8	3			1.6793	
group 1	3			1.7950	

group 2	3				2.4850
Sig.		1.000	.260	.071	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

PROTEINCARBONYL

Duncan[a]

GROUP	N	Subset for alpha = 0.05			
		1	2	3	4
group 5	3	1.4390			
group 7	3	1.9187	1.9187		
group 1	3	2.2140	2.2140	2.2140	
group 4	3	2.2260	2.2260	2.2260	
group 8	3	2.3273	2.3273	2.3273	
group 3	3		2.6420	2.6420	
group 6	3		3.0297	3.0297	
group 9	3			3.2140	
group 2	3				4.3720
Sig.		.107	.050	.076	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

GST

Duncan[a]

GROUP	N	Subset for alpha = 0.05		
		1	2	3

group 2	3	2.2017		
group 7	3		6.4363	
group 3	3		6.5160	
group 5	3		7.0250	
group 9	3		7.3450	
group 4	3		7.4340	
group 8	3		7.7250	
group 6	3		9.4810	
group1	3			16.0513
Sig.		1.000	.102	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

INTERLEUKIN

Duncan[a]

		Subset for alpha = 0.05						
GROUP	N	1	2	3	4	5	6	7
group 5	3	3.8000						
group 6	3	4.0000						
group 7	3		7.2000					
group 4	3		7.4000					
group 8	3			9.6000				
group 1	3				13.3000			
group 3	3					17.0000		
group 9	3						32.4000	
group 2	3							54.4000
Sig.		.730	.730	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

5 ALPHA REDUCTASE OPTIMUM CONCENTRATION

Duncan[a]

GROUP	N	Subset for alpha = 0.05				
		1	2	3	4	5
group 1	3	.5000				
group 6	3		1.2000			
group 4	3			1.5000		
group 8	3			1.7000	1.7000	
group 9	3			1.7000	1.7000	
group 3	3			1.7000	1.7000	
group 5	3			1.8000	1.8000	
group 7	3				2.0000	
group 2	3					2.5000
Sig.		1.000	1.000	.056	.056	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

TNF ALPHA

Duncan[a]

GROUP	N	Subset for alpha = 0.05					
		1	2	3	4	5	6
group 7	3	14.0000					
group 8	3	16.0000					
group 6	3		23.0000				

group 4	3		24.0000				
group 1	3		28.0000	28.0000			
group 9	3			31.0000	31.0000		
group 5	3				35.0000		
group 3	3					50.0000	
group 2	3						59.0000
Sig.		.507	.126	.324	.193	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

TNF BETA

Duncan[a]

		Subset for alpha = 0.05						
GROUP	N	1	2	3	4	5	6	7
group 1	3	13.8000						
group 7	3		38.3333					
group 4	3			62.6900				
group 3	3				67.9533			
group 6	3				70.9300			
group 9	3				70.9300			
group 8	3					76.7600		
group 5	3						81.8400	
group 2	3							88.2400
Sig.		1.000	1.000	1.000	.158	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

DHT

Duncan[a]

GROUP	N	Subset for alpha = 0.05			
		1	2	3	4
group 7	3	5.3000			
group 6	3		7.1000		
group 8	3		7.2000		
group 5	3		8.1000	8.1000	
group 1	3		8.2000	8.2000	
group 9	3			8.4000	
group 3	3			8.7000	
group 4	3			9.1000	
group 2	3				10.5000
Sig.		1.000	.061	.093	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

PROLACTIN

Duncan[a]

GROUP	N	Subset for alpha = 0.05	
		1	2
group 9	3	.8000	
group 4	3	.9000	
group 7	3	.9000	
group 1	3	1.0000	
group 3	3	1.0000	
group 6	3	1.0000	

group 8	3	1.0000	
group 5	3	1.1000	
group 2	3		1.8000
Sig.		.120	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

TESTOSTERONE

Duncan[a]

GROUP	N	Subset for alpha = 0.05					
		1	2	3	4	5	6
group 1	3	2.3000					
group 3	3		6.4000				
group 5	3			7.3000			
group 9	3			7.9000	7.9000		
group 6	3				8.3000	8.3000	
group 8	3					8.7000	
group 7	3						9.6000
group 4	3						9.8000
group 2	3						10.2000
Sig.		1.000	1.000	.075	.224	.224	.089

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

PSA

Duncan[a]

GROUP	N	Subset for alpha = 0.05		
		1	2	3
group 8	3	.1000		
group 1	3	.2000	.2000	
group 7	3	.2000	.2000	
group 9	3	.2000	.2000	
group 5	3		.3000	.3000
group 4	3		.3000	.3000
group 3	3			.4000
group 2	3			.4000
group 6	3			.4000
Sig.		.276	.284	.284

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

```
DATASET NAME DataSet1
WINDOW=FRONT.
ONEWAY CASPASE_PERCENTAGE
PCNA_PERCENTAGE BY GROUP

  /STATISTICS DESCRIPTIVES

  /MISSING ANALYSIS
  /POSTHOC=DUNCAN
ALPHA(0.05).
```

Oneway

Post Hoc Tests

Homogeneous Subsets

CASPASE_PERCENTAGE

Duncan[a]

GROUP	N	Subset for alpha = 0.05						
		1	2	3	4	5	6	7
2	3	5.0000						
1	3		32.0000					
9	3			60.6667				
5	3			62.0000				
6	3				66.6667			
8	3					75.0000		
3	3						80.0000	
7	3						80.0000	
4	3							85.0000
Sig.		1.000	1.000	.550	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

PCNA_PERCENTAGE

Duncan[a]

GROUP	N	Subset for alpha = 0.05					
		1	2	3	4	5	6
1	3	7.0000					
4	3		25.0000				
7	3		25.0000				
3	3			36.0000			
5	3			40.0000	40.0000		
8	3				42.6667		
6	3					55.6667	
9	3					60.0000	
2	3						70.0000
Sig.		1.000	1.000	.158	.339	.128	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

```
NEW FILE.
DATASET NAME DataSet1
WINDOW=FRONT.
ONEWAY
PERCENTAGE_PROSTRATE_
INCRASE_INHIBITION BY
GROUP
  /STATISTICS
DESCRIPTIVES

  /MISSING ANALYSIS
```

```
  /POSTHOC=DUNCAN
ALPHA(0.05).
```

Oneway

Post Hoc Tests

Homogeneous Subsets

PERCENTAGE_PROSTRATE_INCREASE_INHIBITION

Duncan[a]

GROUP	N	Subset for alpha = 0.05	
		1	2
GROUP 7	3	5.6067	
GROUP 9	3	27.7267	27.7267
GROUP 6	3	29.7933	29.7933
GROUP 8	3	32.4500	32.4500
GROUP 4	3	41.0033	41.0033
GROUP 5	3	48.0833	48.0833
GROUP 3	3		69.3233
Sig.		.150	.158

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Oneway

ANOVA

TESTOSTERONE_CONC

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	32.893	3	10.964	411.167	.000
Within Groups	.213	8	.027		
Total	33.107	11			

Post Hoc Tests

Homogeneous Subsets

TESTOSTERONE_CONC

Duncan[a]

SAMPLE	N	Subset for alpha = 0.05			
		1	2	3	4
PSB	3	9.2333			
PSC	3		9.9000		
PSA	3			11.6333	
FINASTERIDE	3				13.5000
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Key: PS_A – Plant sample A (soursop juice)

PS_B – Plant sample B (onion juice)

PS_C – Plant sample C (equal volume combined juice)

Oneway

Post Hoc Tests

Homogeneous Subsets

%_CHANGE_WK1

Duncan[a]

GROUP	N	Subset for alpha = 0.05		
		1	2	3
GROUP 2	3	.7804		
GROUP 9	3	3.5409	3.5409	
GROUP 3	3	4.2425	4.2425	4.2425
GROUP 7	3	4.8260	4.8260	4.8260
GROUP 6	3	4.8784	4.8784	4.8784
GROUP 8	3	4.9905	4.9905	4.9905
GROUP 4	3	6.1340	6.1340	6.1340
GROUP 5	3		8.8524	8.8524
GROUP 1	3			9.7019
Sig.		.062	.064	.057

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

%_CHANGE WK2

Duncan[a]

GROUP	N	Subset for alpha = 0.05	
		1	2
GROUP 8	3	-1.5443	
GROUP 2	3	-.9982	
GROUP 9	3	-.9835	
GROUP 3	3	.1837	
GROUP 7	3	.7372	
GROUP 4	3	2.9555	
GROUP 6	3	4.6204	4.6204
GROUP 5	3	4.7532	4.7532
GROUP 1	3		9.9777
Sig.		.071	.094

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

%_CHANGE_WK3

Duncan[a]

GROUP	N	Subset for alpha = 0.05			
		1	2	3	4
GROUP 2	3	-4.6721			
GROUP 8	3	-2.7468	-2.7468		
GROUP 7	3	-2.4407	-2.4407		
GROUP 9	3	-.1792	-.1792	-.1792	
GROUP 5	3	.4718	.4718	.4718	.4718
GROUP 6	3	1.9191	1.9191	1.9191	1.9191
GROUP 4	3		3.3180	3.3180	3.3180
GROUP 3	3			4.7287	4.7287
GROUP 1	3				7.1335
Sig.		.055	.076	.141	.050

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

%_CHANGE_WK4

Duncan[a]

GROUP	N	Subset for alpha = 0.05	
		1	2
GROUP 7	3	-2.0640	
GROUP 5	3	1.8915	1.8915
GROUP 6	3	2.9288	2.9288
GROUP 9	3	3.0716	3.0716
GROUP 1	3	3.0990	3.0990
GROUP 4	3	4.4020	4.4020
GROUP 8	3	4.6857	4.6857

GROUP 2	3		5.2247
GROUP 3	3		5.2994
Sig.		.063	.333

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

AVERAGE_CHANGE_IN _WEIGHT

Duncan[a]

		Subset for alpha = 0.05		
GROUP	N	1	2	3
GROUP 2	3	.0008		
GROUP 7	3	.0026		
GROUP 8	3	.0135	.0135	
GROUP 9	3	.0136	.0136	
GROUP 6	3		.0359	
GROUP 3	3		.0361	
GROUP 5	3		.0399	
GROUP 4	3		.0420	
GROUP 1	3			.0748
Sig.		.417	.088	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

AVERAGE_%_CHANGE_IN_WT

Duncan[a]

		Subset for alpha = 0.05		
GROUP	N	1	2	3
GROUP 2	3	.0837		
GROUP 7	3	.2646		
GROUP 8	3	1.3463	1.3463	
GROUP 9	3	1.3625	1.3625	
GROUP 6	3		3.5867	
GROUP 3	3		3.6136	
GROUP 5	3		3.9922	
GROUP 4	3		4.2024	
GROUP 1	3			7.4780
Sig.		.417	.088	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.