ANTIOXIDANT EFFECT OF SEED EXTRACT OF *Persea americana* IN BENIGN PROSTATE HYPERPLASIA RATS AND LC-MS PROFILING OF IT'S METABOLITES

BY

AJAYI, VICTOR OPEYEMI

PHA 1305003

DEPARTMENT OF PHARMACOGNOSY,

FACULTY OF PHARMACY,

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A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF PHARMACOGNOSY, FACULTY OF PHARMACY, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHARMACY (PHRAM.D.) DEGREE,

UNIVERSITY OF BENIN,

BENIN CITY.

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CERTIFICATION

This research project titled "Antioxidant effect of ethanol seed extract of Persea americana in Benign Prostate Hyperplasia Rats and spectroscopic identification of associated secondary metabolites" is an original research work carried out by AJAYI, VICTOR OPEYEMI, under the supervision of Dr. (Mrs.) Josephine Ofeimun, in the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

_____ _____

Dr. (Mrs.) J. Ofeimun. **Date**
(Project Supervisor)

_____ _____

Dr. (Mrs.) J. Ofeimun. **Date**
(Head of Department)

_____ _____

EXTERNAL EXAMINER. **Date**

DEDICATION

I dedicate this work to the Almighty God for His immeasurable love and grace throughout my stay in this institution, to my parents Late Mr. and Mrs. Innocent Ajayi Bello, my uncle Pst. T.A Badakin, and my siblings for their continuous support and motivation.

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I am sincerely grateful to my parents Late Mr. and Mrs. Innocent Ajayi Bello, my siblings, my uncle Pst. T. A. Badakin, my aunt Rev. Sr. Monica Rowland, the Osasonas, Vivian and Ghino Pharmacy Family for the invaluable role they have played in life this far.

Special thanks to my esteemed classmates, colleagues and friends from other departments and walk of life. May God bless you richly.

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ABSTRACT

Benign prostate hyperplasia is a disease of ageing men. Oxidative stress is a promoter of the ageing process. This study evaluated the effect of the ethanol seed extract of *Persea Americana* (PAE) on antioxidant and lipid per oxidation status of male rats induced with benign prostate hyperplasia and spectroscopic analysis of the extract. Five groups of five rats each were induced with benign prostate hyperplasia by the subcutaneous administration of testosterone (3mg/kg). Group 1 rats (negative control) received 10 ml/kg of distilled water. Group 2 animals (standard control) were treated with finasteride (4mg /kg). Groups 3, 4 and 5 received 100, 200 and 400 mg/kg dose of PAE respectively. Groups 6 animals (normal control) were neither induced nor treated. All administration was daily for 28 days by oral gavage. Rats were sacrificed on the 29th day, blood obtained, and serum enzyme activities of superoxide dismutase (SOD), catalase (CAT), glutathione reductase (Gsr) and glutathione peroxidase (GPx) were measured with Elisa assay test kits. Malondialdehyde concentration was equally measured. High-Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS) was used to identify compounds present in PAE. The extract significantly ($P<0.0001$) increased the activities of SOD, CAT, GPx and Grs compared to the negative control at the dose of 400 mg/kg while MDA concentration was significantly reduced. Digoxin, tetramethylquercetin and abscisic acid were identified in PAE. The potential of the extract of PAE to ameliorate testosterone-induced BPH through enhancement of antioxidant defense system is demonstrated by this study.

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

The use of plants as a source of remedies for treating various illnesses is as old as mankind with recorded practices going back at least 4000 years (Christophersen *et al*., 1991). Although Nigeria has a rich and long tradition in the use of medicinal plants (Daiziel, 1937) their systematic study has just recently been started.

The undesirable acute, chronic and toxic effects, the long and tedious processes involved in the synthesis of synthetic drugs as well as other shortcomings, have led to the search for alternative sources with a view to obtaining drugs which have greater advantages and lesser disadvantages.

This has led to the turn on of the search light towards drugs obtained from plant sources. Some of the desirable advantages which these plant sources of drugs possess include;

a) Their ability to yield compounds with unique activity. (Braquet *et al*., 1991)

b) Their use in chronic condition for which conventional medicine is preceived to offer little (Sherman *et al*., 1993)

c) Inexpensive sources of "feedstock" molecule that can steadily be transformed into drugs (Kingorn *et al*., 1993)

d) Alternative to established therapy because they act at different stages of the disease and thus useful in combination therapy (Waigh, 1988).

e) Compounds from plants with less driect therapeutic potential may offer new molecule templates for the design of more effective drugs (Stress *et al*., 1990)

Until the 1950s, almost all pharmaceutical research relied mainly on vascular plants as source of medicine (Cox and Balick, 1994). Flowering plants and ferns (as opposed to microscope organisms and fungi) have given rise to about one hundred and twenty commercially sold drugs and account for some twenty five percent of all plant prescription issued every year in North America (Cox and Balick., 1994).

Many of these agents are now synthesized, in the laboratory but others are still isolated from plants. Most were discovered by studying indigenous uses of plants.

Even with these seeming advances in the area of research, facts have shown that this area is not well utilized. It is estimated that 265,000 flowering plant species grace the earth, of which less than one percent have been studied exhaustively for their chemical composition and medicinal value (Cox and Balick., 1994). Also less than ten percent of estimated number of flowering species in the world has been examined scientifically for their potential in medicine. It has been suggested that about 60,000 species of higher plants (i.e. 1 in 4) will probably have become extinct by the year 2050 (Braquet *et al*., 1991). This accelerating loss of species resulting from destruction of plants with the attrition of indigenous cultures, strikes a note of urgency into the quest for new compounds of therapeutic interest (Akerale *et al.*, 1991).

Ethnopharmacology is that scientific study of plants used by a cultural group medicial purposes. This special area of study needs to be intensified with priority to chronic diseases, for which

current medications have several drawbacks and to the scientific appraisal of plant based remedies that might be cheaper, safer and less toxic than existing prescribable ones. The need for this study is evident from the above factors and is a pointer that a lot of work has to be done in that direction.

1.2 PROSTATIC HYPERPLASIA

Benign prostatic hyperplasia (BPH) is a non-cancerous enlargement of the prostate gland. It is the most common benign tumor found in men. About 50% of males over the age 50 and about 90% of males over the age 80 suffer from benign prostatic hyperplasia symptoms. (Kang *et al*., 2017).

Benign prostatic hyperplasia (BPH) is a significant cause of urinary symptoms in the adult male. Enlargement of the prostate occurs with age leading to bladder out-let obstruction, which manifest with symptoms of impaired urine voiding and/or storage, referred to as lower urinary tract symptoms (LUTS). Although BPH is not life threatening it's clinical manifestations as LUTS reduces the patient's quality of life. (Ojewola *et al.*, 2017).

Generally, the androgen receptor (A) signaling pathway is known to play a crucial role in the occurance of BPH. (Izumi *et al*., 2013). Even though the exact mechanism of BPH still needs to be investigated, the close relation between BPH and androgen. The hormonal Cascade starting by the action of 5-alpha-reductase (5AR), is known to be one of the pathways responsible for the pathogenesis of BPH.

1.2.1 AETIOLOGY OF BENIGN PROSTATE HYPERPLASIA

It is known that testosterone produced in the testis spreads to the prostate and is converted to dihydrotestosterone (DHT) by 5-alpha-reductase (5AR), an enzyme involved in stored

metabolism, to regulate the development of BPH. (Thigpen *et al.*, 1993; Asada *et al*., 2001). The over supply of testosterone leads to an excessive DHT conversion in the prostate via action of 5AR. (Clark *et al*., 2004).

This converted DHT has a much higher binding ability to androgen receptor (AR) compared to testosterone (Andriole *et al*., 2004). In prostate cells, testosterone or DHT combine to AR, leading to increases in transcription of androgen- dependent genes, and as a result, proliferation is stimulated. (Rochroborn 2008). Therefore, the pathogenesis of BPH is closely related to the 5AR- AR axis. In this process, DHT enhance prostate specific antigen (PSA) levels. PSA levels can be raised by enlarged prostate volume or acute inflammation. Even though PSA is not a unique index for prostate cancer, PSA is widely used to help diagnose BPH. (Kang *et al*., 2017).

Estrogen is a female hormone that is commonly known to play a major role in the development of secondary sexual characteristics in women, but it is also, an important hormone of men. Estrogen is reported to stimulate prostate growth, and it is associated with the development of prostate disease in men. However, estrogen receptor- alpha (ER alpha) and estrogen receptor- beta (ER beta) have different roles. Because ER alpha mediates increasing of cell proliferation and ER beta mediates apoptosis. ER beta positively affect BPH, but ER alpha has a negative effect on BPH (Kang *et al*., 2017).

Oxidative Stress has been considered to be one of the mechanisms that triggers the development and progression of BPH. Levels of antioxidants, including zinc, are decreased in prostatic hyperplasia. In addition, animal models with BPH have shown increased prostatic lipid peroxidation and reduced endogenous antioxidants (eg glutathione, superoxide dismutase and catalase) (Yana *et al*., 2010).

Chronic inflammation is a common finding in BPH. A significant correlation has been found between prostate size and both acute and chronic inflammation and data suggest that this could be a causative effect, rather than occurring simply as a consequence. Potential causes include infectious agents, environmental factors (toxins), diet, lifestyle, hormonal or metabolic imbalances or a combination of these. Low testosterone is also associated with increased inflammatory mediators; and there have been a number of studies that show a link between decreased DHT levels and increased inflammation (Vignozzi *et al*., 2012).

1.2.2 EPIDEMIOLOGY

Globally, BPH affects about 210 million males as of 2010 (6% of the population) (Vos, *et al*., 2012). The prostate gets larger in most men as they get older. For a symptom-free man of 46 years, the risk of developing BPH over the next 30 years is 45%. Incidence rates increase from 3 cases per 1000 man-years at age 45–49 years, to 38 cases per 1000 man-years by the age of 75–79 years. While the prevalence rate is 2.7% for men aged 45–49, it increases to 24% by the age of 80 years (Verhamme *et al*., 2002).

1.2.3 SIGNS AND SYMPTOMS

BPH is the most common cause of lower urinary tract symptoms (LUTS), which are divided into storage, voiding, and symptoms which occur after urination. Storage symptoms include the need to urinate frequently, waking at night to urinate, urgency (compelling need to void that cannot be deferred), involuntary urination, including involuntary urination at night, or urge incontinence (urine leak following a strong sudden need to urinate). Voiding symptoms include urinary hesitancy (a delay between trying to urinate and the flow actually beginning), intermittency (not continuous), involuntary interruption of voiding, weak urinary stream, straining to void, a

sensation of incomplete emptying, and uncontrollable leaking after the end of urination. (Robinson, 2008; Sarma and Wei, 2012). These symptoms may be accompanied by bladder pain or pain while urinating, called dysuria.

Bladder outlet obstruction (BOO) can be caused by BPH. Symptoms are abdominal pain, a continuous feeling of a full bladder, frequent urination, acute urinary retention (inability to urinate), pain during urination (dysuria), problems starting urination (urinary hesitancy), slow urine flow, starting and stopping (urinary intermittency), and nocturia.

BPH can be a progressive disease, especially if left untreated. Incomplete voiding results in residual urine or urinary stasis, which can lead to an increased risk of urinary tract infection. (Truzzi *et a*l., 2008).

1.2.4 CAUSES

1.2.4a Hormone

Most experts consider androgens (testosterone and related hormones) to play a permissive role in the development of BPH. This means that androgens must be present for BPH to occur, but do not necessarily directly cause the condition. This is supported by evidence suggesting that castrated boys do not develop BPH when they age. In an unusual study of 26 eunuchs from the palace of the Qing dynasty still living in Beijing in 1960, the prostate could not be felt in 81% of the studied eunuchs. The average time since castration was 54 years (range, 41–65 years) (Wu and Gu, 1991).

Testosterone promotes prostate cell proliferation (Feldman and Feldman, 2001), but relatively low levels of serum testosterone are found in patients with BPH (Legiou *et al*., 1997; Roberts *et al.,* 2004). One small study has shown that medical castration lowers the serum and prostate

hormone levels unevenly, having less effect on testosterone and dihydrotestosterone levels in the prostate (Paga *et al*., 2006).

Dihydrotestosterone (DHT), a metabolite of testosterone, is a critical mediator of prostatic growth. DHT is synthesized in the prostate from circulating testosterone by the action of the enzyme 5α-reductase, type 2. DHT can act in an autocrine fashion on the stromal cells or in paracrine fashion by diffusing into nearby epithelial cells. In both of these cell types, DHT binds to nuclear androgen receptors and signals the transcription of growth factors that are mitogenic to the epithelial and stromal cells. DHT is ten times more potent than testosterone because it dissociates from the androgen receptor more slowly. The importance of DHT in causing nodular hyperplasia is supported by clinical observations in which an inhibitor of 5α-reductase such as finasteride is given to men with this condition. Therapy with a 5α-reductase inhibitor markedly reduces the DHT content of the prostate and, in turn, reduces prostate volume and BPH symptoms.(Bartsch *et al*., 2002).

While there is some evidence that estrogen may play a role in the cause of BPH, this effect appears to be mediated mainly through local conversion of androgens to estrogen in the prostate tissue rather than a direct effect of estrogen itself (Ho *et al*., 2008). Studies looking for a correlation between prostatic hyperplasia and serum estrogen levels in humans have generally shown none (Roberts *et al*., 2004; Ansari *et al*., 2008).

1.2.4b Diet

Studies indicate that dietary patterns may affect development of BPH, but further research is needed to clarify any important relationship.(Heber, 2002). Studies from China suggest that greater protein intake may be a factor in development of BPH. Men older than 60 in rural areas

had very low rates of clinical BPH, while men living in cities and consuming more animal protein had a higher incidence. (Zheng *et al*., 2003; Gu, 1997). On the other hand, a study in Japanese-American men in Hawaii found a strong negative association with alcohol intake, but a weak positive association with beef intake (Chyou *et al*., 1993).

1.2.4c Degeneration

BPH is an age-related disease. Misrepair-accumulation aging theory (Wang *et al*., 2009; Wang-Michelitsch and Michelitsch, 2012) suggests that development of benign prostatic hyperplasia is a consequence of fibrosis and weakening of the muscular tissue in the prostate. (Wang-Michelitsch and Michelitsch, 2012). The muscular tissue is important in the functionality of the prostate, and provides the force for excreting the fluid produced by prostatic glands. However, repeated contractions and dilations of myofibers will unavoidably cause injuries and broken myofibers. Myofibers have a low potential for regeneration; therefore, collagen fibers need to be used to replace the broken myofibers. Such misrepairs make the muscular tissue weak in functioning, and the fluid secreted by glands cannot be excreted completely. Then, the accumulation of fluid in glands increases the resistance of muscular tissue during the movements of contractions and dilations, and more and more myofibers will be broken and replaced by collagen fibers.

1.2.5 PATHOPHYSIOLOGY

As men age, the enzymes aromatase and 5-alpha reductase increase in activity. These enzymes are responsible for converting androgen hormones into estrogen and dihydrotestosterone, respectively. This metabolism of androgen hormones leads to a decrease in testosterone but increased levels of DHT and estrogen.

Both the glandular epithelial cells and the stromal cells (including muscular fibers) undergo hyperplasia in BPH. (Kim *et al*., 2016).

Anatomically the median and lateral lobes are usually enlarged, due to their highly glandular composition. The anterior lobe has little in the way of glandular tissue and is seldom enlarged. (Carcinoma of the prostate typically occurs in the posterior lobe – hence the ability to discern an irregular outline per rectal examination). In BPH, the majority of growth occurs in the transition zone (TZ) of the prostate. In addition to these two classic areas, the peripheral zone (PZ) is also involved to a lesser extent. (Wesserman, 2006).

BPH can be a progressive growth that in rare instances leads to exceptional enlargement. In some males, the prostate enlargement exceeds 200 to 500 grams. This condition has been defined as giant prostatic hyperplasia (GPH) (Üçer, 2011).

1.2.6 DIAGNOSIS

The clinical diagnosis of BPH is based on a history of LUTS (lower urinary tract symptoms), a digital rectal exam, and exclusion of other causes of similar signs and symptoms. The degree of LUTS does not necessarily correspond to the size of the prostate. An enlarged prostate gland on rectal examination that is symmetric and smooth supports a diagnosis of BPH. However, if the prostate gland feels asymmetrical, firm, or nodular, this raises concern for prostate cancer. (Kim, *et al*., 2016).

Urinalysis is typically performed when LUTS are present and BPH is suspected to evaluate for signs of a urinary tract infection, glucose in the urine (suggestive of diabetes), or protein in the urine (suggestive of kidney disease) (Kim *et al*., 2016).

Blood test including kidney function tests and prostate specific antigen (PSA) are often ordered to evaluate for kidney damage and prostate cancer, respectively. However, checking blood PSA levels for prostate cancer screening is controversial and not necessarily indicated in every evaluation for BPH. Benign prostatic hyperplasia and prostate cancer are both capable of increasing blood PSA levels and PSA elevation is unable to differentiate these two conditions well. If PSA levels are checked and are high, then further investigation is warranted. Measures including PSA density, free PSA, rectal examination, and transrectal ultrasonography may be helpful in determining whether a PSA increase is due to BPH or prostate cancer. Ultrasound examination of the testes, prostate, and kidneys is often performed, again to rule out cancer and hydronephrosis. (Kim *et al*., 2016).

1.2.7 MANAGEMENT.

When treating and managing benign prostatic hyperplasia, the aim is to prevent complications related to the disease and improve or relieve symptoms. (Hwang *et al.*, 2018). Approaches used include lifestyle modifications, medications, and surgery.

1.2.7a Lifestyle.

Lifestyle alterations to address the symptoms of BPH include physical activity, (Silva *et al*., 2019) decreasing fluid intake before bedtime, moderating the consumption of alcohol and caffeine-containing products and following a timed voiding schedule.

1.2.7b Physical activity.

Physical activity has been recommended as a treatment for urinary tract symptoms. A 2019 Cochrane review of six studies involving 652 men assessing the effects of physical activity alone, physical activity as a part of a self-management program, among others. However, the quality of

evidence was very low and therefore it remains uncertain whether physical activity is helpful in men experiencing urinary symptoms caused by benign prostatic hyperplasia. (Silva *et al*., 2019).

1.2.7c Voiding position.

Voiding position when urinating may influence urodynamic parameters (urinary flow rate, voiding time, and post-void residual volume). A meta-analysis found no differences between the standing and sitting positions for healthy males, but that, for elderly males with lower urinary tract symptoms, voiding in the sitting position. (De Jong *et al*., 2014)

- decreased the post void residual volume
- increased the maximum urinary flow, comparable with pharmacological intervention
- decreased the voiding time

1.2.7c Medications.

The two main medication classes for BPH management are alpha blockers and 5α-reductase inhibitors. (Silva *et al*., 2014).

i. Alpha blockers.

Selective alpha-1 blockers are the most common choice for initial therapy.(Roehrbron *et al*., 2007; Black *et a*l., 2006). They include alfuzosin, (MacDonald and Wilt, 2005) doxazosin, silodosin, tamsulosin, terazosin, and naftopidil (Hwang *et al*., 2018). They have a small to moderate benefit at improving symptoms. Selective alpha-1 blockers are similar in effectiveness but have slightly different side effect profiles.(Wilt *et al*., 2013; Djavan and Marberger, 1999) Alpha blockers relax smooth muscle in the prostate and the bladder neck, thus decreasing the blockage of urine flow. Common side effects of alpha blockers include orthostatic hypotension

(a head rush or dizzy spell when standing up or stretching), ejaculation changes, erectile dysfunction, (Santillo and Lowe, 2006) headaches, nasal congestion, and weakness.

ii. 5α-Reductase inhibitors

The 5α-reductase inhibitors finasteride and dutasteride may also be used in men with BPH.(Blankstein *et al*., 2016). These medications inhibit the 5α-reductase enzyme, which, in turn, inhibits production of DHT, a hormone responsible for enlarging the prostate. Effects may take longer to appear than alpha blockers, but they persist for many years. (Roehrborn *et al*., 2004) When used together with alpha blockers, no benefit was reported in short-term trials, but in a longer-term study (3–4 years) there was a greater reduction in BPH progression to acute urinary retention and surgery than with either agent alone, especially in people with more severe symptoms and larger prostates. (Greco and MaVary, 2008; Kaplan *et al*., 2006). Other trials have confirmed reductions in symptoms, within 6 months in one trial, an effect that was maintained after withdrawal of the alpha blocker. (Greco and MaVary, 2006; Barkin *et al*., 2003). Side effects include decreased libido and ejaculatory or erectile dysfunction. (Gromley *et al*., 1992; Gacci *et al*., 2014).

iii. Phosphodiesterase inhibitors (PDE)

A 2018 Cochrane review of sty bother but may also cause more side effects compared to placebo. The evidence in this review found that there is probably no difference between PDE and alpha blockers, however when used in combination they may provide a greater improvement in symptoms (with more side effects). PDE also likely improves symptoms when used in combination with 5-alpha reductase inhibitors.

Several phosphodiesterase-5 inhibitors are also effective, but may require multiple doses daily to maintain adequate urine flow. (Wang *et al*., 2018; Pattanaik *et al*., 2018).

1.2.7d**. SURGERY**

If medical treatment is not effective, surgery may be performed. Surgical techniques used include the following:

Transurethral resection of the prostate (TURP): the gold standard (Hoffman *et al*., 2012). TURP is thought to be the most effective approach for improving urinary symptoms and urinary flow, however, this surgical procedure may be associated with complications in up to 20% of men (Hoffman *et al*., 2012). Surgery carries some risk of complications, such as retrograde ejaculation (most commonly), erectile dysfunction, urinary incontinence, urethral strictures.

Transurethral incision of the prostate (TUIP): rarely performed; the technique is similar to TURP but less definitive.

Open prostatectomy: not usually performed nowadays due to its high morbidity, even if results are very good.

Some less invasive procedures are available according to patients' preferences and co-morbidities. These are performed as outpatient procedures with local anesthesia. They include;

i. Prostatic artery embolization: an endovascular procedure performed in interventional radiology (Kuang *et al*., 2017). Through catheters, embolic agents are released in the main branches of the prostatic artery, in order to induce a decrease in the size of the prostate gland, thus reducing the urinary symptoms (Pisco *et al*., 2016).

ii. Water vapor thermal therapy: This is a newer office procedure for removing prostate tissue using steam aimed at preserving sexual function. They include;

iii. Prostatic urethral lift (marketed as UroLift): This intervention consists of a system of a device and an implant designed to pull the prostatic lobe away from the urethra (McNicholas, 2016).

iv. Transurethral microwave thermotherapy (TUMT) is an outpatient procedure that is less invasive compared to surgery and involves using microwaves (heat) to shrink prostate tissue that is enlarged (Hoffman *et al*., 2012).

v. Temporary implantable nitinol device (TIND and iTIND): is a device that is placed in the urethra that, when released, is expanded, reshaping reshaping the urethra and the bladder neck (Propiglia *et al*., 2015).

1.2.7e Alternative medicine.

While herbal remedies are commonly used, a 2016 review found the herbs studied to be no better than placebo. (Keehn *et al*., 2016) Particularly, several systematic reviews found that Saw palmetto extract from *Serenoa repens*, while one of the most commonly used, is no better than placebo in both symptom relief and decreasing prostate size (Bent *et al*., 2006; Dadhia and MaVary, 2008; Tacklind *et al*., 2012). Other ineffective herbal medicines include beta-sitosterol (Wilt *et al*., 1999) from *Hypoxis rooperi* (African star grass), pygeum (extracted from the bark of *Prunus africana*), (Wilt *et al*., 1998) pumpkin seeds (*Cucurbita pepo*) and stinging nettle (*Urtica dioica*) root (Wilt *et al*., 2000). A systematic review of Chinese herbal medicines found that Chinese herbal medicine, either as monotherapy or an adjuvant therapy with Western medicine, was similar to either placebo or Western medicine in the treatment of BPH. Chinese herbal

medicine was found to be superior to Western medicine in improving quality of life and reducing prostate volume (Ma *et al*., 2013).

Not withstanding the plenty of evidence to support the clinical effectiveness of herbal therapies in the management of BPH, the adverse side effects associated with the use of conventional drugs, invasive nature and economic burden of alternative management option continue to drive the search for herbal therapies that will prove effective in the management of the condition. This has lead to the focus on *Pearsea american* seed, which is claimed in ethnomedicine to be effective in the management of BHP (Ofeimum and Fanatao, 2020)

1.3 Persea americana(Mill)

Persea americana (Mill), a tree likely originating from southcentral Mexico, (Morto, 1987; Chen *et al.*, 2008) is classified as a member of the flowering plant family Lauraceae. (Morto JF, 1987) The fruit of the plant, also called an avocado (or avocado pear or alligator pear), is botanically a large berry containing a single large seed (Storey, 1973). It is cultivated in tropical and Mediterranean climates of many countries including Nigeria.

1.3.1 Scientific classification of *Persea americana*

Kingdom:	Plantae
Division:	Tracheophytes
Sub-division:	Angiosperms
Class:	Magnoliids
Order:	Laurales
Family:	Lauraceae

Genus: *Persea*

Species: *americana*

Common name: Avocado pear

The fruit of domestic varieties has a buttery flesh when ripe. Depending on the variety, avocados have green, brown, purplish, or black skin when ripe, and may be pear-shaped, egg-shaped, or spherical. Commercially, the fruits are picked while immature, and ripened after harvesting.

1.3.2 DESCRIPTION.

Persea americana (Mill), is a tree that grows to 20 m (66 ft), with alternately arranged leaves 12–25 cm (5–10 in) long. Panicles of flowers with deciduous bracts arise from new growth or the axils of leaves. (Dilip N, 2014) The flowers are inconspicuous, greenish-yellow, 5–10 mm (3⁄16–3⁄8 in) wide.

Persea americana seed is encased in a hard shell and comprises of 13 - 18% of the size of the whole fruit. Information about its composition is limited, but it does contain a good range of fatty acids dietary fiber, carbohydrates and small amount of protein. (O'Brien, 2018).

The seed is also considered to be a rich source of phytochemicals, of which some of the phytochemicals may have antioxidant potential, others may offer any health benefits. (O'Brien, 2018). It has been established that *Persea americana* seed extract have antihypertensive effect, as it is has been used especially in Nigeria, in the management of high blood pressure.

1.3.3 ETHNOMEDICINAL USES

Persea americana (Mill.) has for long been used traditionally in the treatment of many ailments known to man in different parts of the world. The root, bark, fruit, seed and leaf are used in traditional medicine in many countries. The stem bark and the leaves are boiled together in water, with the resulting fluid taken to cure toothache, malaria and typhoid fever (Tchaghebe *et al*., 2016).

In Cameroon, leaves decoction of *Persea americana* is taken for the treatment of toothache, high blood pressure, diabetes and malaria. It is also used to stimulate uterine contraction and relief painful menstruation. Also the leaves have been popularly used in the Latin America and Africa for the treatment of diabetes (Lima *et al.*, 2012).

In Cuba, *Persea americana,* is used as antitussive, carminative, anti-diarrheal, abortifacient and stomachic, also it was reported to be indicated in cases of amenorrhea, liver obstruction, influenza and excess uric acid (Roig *et al*., 1998).

The seed of the plant is used in Mexican traditional medicine to treat skin rashes, diarrhea and dysentery caused by helminthes and amoebas. It is also used to cure infection of fungal and bacterial origin, as well as in the treatment of asthma, high blood pressure and rheumatism (Agular *et al*., 1994; Argute *et al*., 1994; Refugio *et al*., 2004). The seed is also used in beautification (Pumplora *et al*., 1999). The seed of the plant has been reported to used used in the management of BPH by the people of southwestern Nigeria (Ofemun and Fanatao, 2020).

1.3.4 PHARMACOLOGICAL ACTIVITY

The research data available on *Persea americana* indicate that it possesses several Pharmacological uses, which supports its multiple traditional uses for management of different health problems, some of them are:

1.3.4a. Anti-malaria activity

Falodun *et al* (2014), studied the anti-malaria activities of *Persea americana* leaf extract and reported that the plant leaf extract contain fatty alcohol metabolite that possess potent activity against chloroquine sensetive and chloroquine resistant strains of *Plasmodium falciparum.* This evidence was corroborated by Kamlaga *et al* (2015), who equally reported that aqueous extract of the leaf possesses anti-malaria activities.

1.3.4b Antiulcer and gastro protective activity

Aqueous leaf extract of *Persea americana* have equally been reported to effect in significant dose-dependent antiulcer activity when administrated orally to sick rats (rats pre-treated with indomethacin and ethanol) (Ukwe and Nwafor, 2004).

1.3.4c Anti convulsion activity

Aqueous leaf extract of *Persea americana* has been found to possess anticonvulsant activity as it antagonized seizures induced in mice by the administration of the drug viz, pentylenetriazole (PTZ) and picotoxin (PCT) (Ojwole *et al*., 2006).

1.3.4dAntioxidant activity

The antioxidant properties of the leaves and seed of *P. americana* have equally been reported by Owusu-Boadi *et al* (2015) and Ikpeme *et al* (2014) respectively. The antioxidant activity of the fresh was determined to be higher than that of dried fruit.

1.3.4e Antidiabetic activity

Thenmozhi *et al*, (2012) carried out a study to determine the most effective fraction and subfraction hydro-mathanolic extract of *Persea americana* fruit on hypoglycemic effect on streptozotocin-induced diabetic rats. The result obtained show that a subfraction obtained from n-hexane fraction was most effective. In addition, a study has demonstrated that aqueous leaf extract of *Persea americana* possesses hypoglycemic effect on normal rats, with the maximum effect achieved at 6 hours after oral administration of a single dose (Anita *et al*., 2005).

1.3.4f Vasorelaxant activity

The vasorelaxant properties of aqueous leaf extract of *Persea americana* on isolated rat aorta has been investigated. A significant vasorelaxation in aorta has been observed due to the synthesis of endothelium derived relaxing factors (EDRF's) and the release of prostanoid. The treatment of the aorta with the extract equally reduced vasoconstriction, the probable reason for it being the inhibition of Ca2+ influx through calcium channel (Owolabi *et al*., 2005).

1.3.4.g Effect on body weight

It is evident from Brail *et al's* study that the administration of aqueous and methanol leaf extracts of *Persea americana* caused a reduction in body weight compared with the hyperlipidemic controls (Brail *et al*., 2007). It is believe that *Persea americana* leaf extracts

increase the catabolism of lipids accumulated in the adipose tissue, leading to a decrease in the mean body weight (Tene *et al*., 2016).

1.3.4h Antibacterial and antimicrobial activity

Many studies have shown that the *P. americana* possesses antibacterial and antimicrobial properties. In a study on protozoal and antimycobacterial activities of *P. americana* seeds, the chloroform and ethanolic extracts of the seed showed significant activity against *Entamoeba histolytica, Giardia lamblia, Trichomonas vaginalis.* The seed extract is also active against *Escherichia coli, Klebsiella americana, Bacillus subtilis, Streptococcus pyrogenes, Pseudomonas aeruginosa, Staphylococcus aureus, Corynebacterium ulcerans, Salmonella typhi, Neisseria americana and Candida albicans* (Idris *et al*., 2009).

1.3.4i Antiviral activity

The infusion and ethanol extract of dried leaves of *Persea americana* were compared with respect to their inhibitory activities on viral replication in vitro. The chosen viruses for the initial screening were adenovirus type 3 (AD3), HSV-1, and ADV. The ethanol extract was only tested against HSV-1 and ADV. The infusate was active against the 3 viruses, whereas the ethanol extract did not show any activity under the experimental conditions employed (de Almeida *et al*., 1998).

1.3.4j Wound healing activity

The wound healing properties of the *P. americana* fruit extract has been reported by Nayak *et al* (2008). The topical and oral administration of the fruit extract to wounded rats resulted in the complete healing of the wound. Other parameters like rate of wound contraction and hydroxyproline content of tissues along healing with histological observations

also indicated the wound healing property of the plant. In the excision wound model, complete healing (full epithelialization) was observed on average on day 14 in the rats, which received oral or topical treatment of the fruit extract (300 mg/kg/day) (Carvajai-Zarrabai *et al*., 2014).

1.3.4k Antihepatotoxic activity

The methanolic extract of *P. americana* have been demonstrated to be protective against toxicity and oxidative stress arising from acute paracetamol intoxication. The mechanism of this protection is thought to be due to an antioxidant action of catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase, which are the primary intracellular defense mechanisms to cope with increased oxidant stress. The activities of the antioxidant enzymes measured (SOD and CAT) did not change significantly in normal rats treated with the extract, however, the extract significantly induced the activity of these enzymes during hepatic damage produced by acute paracetamol toxicity. (Ekor *et al*., 2006).

1.3.4l Anticancer activity

Avocado seeds contain a flavonoid that prevents tumor growth (Omodamiro *et al*., .2016). It has been also reported that the lipophilic carotenoids present in this plant extract may have potential anti-carcinogenic effects (Ding *et al*., 2007). In another study, the compound, persin, isolated from the leaves of *P. americana* has been used to carry out the induction of apoptosis in human breast cancer cells

1.3.5 PHYTOCHEMISTRY

Different classes of secondary metabolites have been reported to be present in *P. americana*these include alkanols (also sometimes termed “aliphatic acetogenins”), terpenoid glycosides, various furan ring-containing derivatives, flavonoids, and coumarin. Oberlies et al (1998) isolated 1,2,4-trihydroxyheptadec-16-ene, 1,2,4-trihydroxyheptadec-16-yne, and 1,2,4-trihydroxynonadecane from the unripe fruits of *Persea americana,* and found these substances to be moderately cytotoxic when evaluated against a small panel of cancer cell lines. Persin [15; (2R,12Z,15Z)-2-hydroxy-4-oxoheneicosa-12,15-dienyl acetate], a constituent of avocado leaves, is regarded as a toxin for lactating livestock (Oelrich *et al*., 1995).Related compounds, namely; persenones A and B, have been identified in the leaves. (Domergue *et al*., 2000).

The glycosylated abscisic acid derivatives (1S,6R)-8-hydroxy abscisic acid-d-glucoside and (1R,3R,5R,8S)-pi-dihydrophaseic acid-d-glucoside were isolated from the seeds of *Persea americana*(del Rafugio-Ramos, et. al., 2004).

Furanoid constituents of avocados have been isolated and structurally characterized or chemically synthesized by several different groups (Kashman, et. al., 1969; Morakoshi, et. al., 1976; Rodriguez-Saona *et al*., 2000). Flavonoids, quercetin, afzelin and quercetin 3-O-d have been isolated from the leaves and seeds of P. americana.

Scopoletin, a coumarin, have been isolated from the leaves of the plant. (Merici *et al*., 1993), while, (E,Z,Z)-1-Acetoxy-2-hydroxy-4-oxo-heneicosa-5,12,15-triene, was isolated from the idioblast of *P. americana.*

1.3.6 IDENTIFIED AND ISOLATED COMPOUNDS FROM *PRESEA AMERICANA*

A number of compounds belonging to different chemical groups have either isolated or identified from various parts and extract/fractions of P. american. These include; 1,2,4-trihydroxyheptadec-16-ene, 1,2,4-trihydroxyheptadec-16-yne, and 1,2,4-trihydroxynonadecane from the unripe fruits (Overlies *et al*.,1998). Equally, Persenone-C , Persenone-A (Rodriguez-Sanchez *et al*., 2014).

The antifungal compound (E,Z,Z)-1-Acetoxy-2-hydroxy-4-oxo-heneicosa-5,12,15-triene, was isolated from the idioblast of *Persea Americana* (Mill.). This compound inhibits pore germination of the fungal pathogen *Colletotrichum gloeosporioides* (Ekor *et al*., 2006).

Compounds have also been isolated from the seed of the plant, they include, thehe glycosylated abscisic acid derivatives (1S,6R)-8-hydroxy abscisic acid-d-glucoside and (1R,3R,5R,8S)-pi-dihydrophaseic acid-d-glucoside (del Rafugio-Ramos *et al*., 2004). Also, some bioactive phenolic compounds have been identified from the ethanolic extract of the seed, such as, catechin, epicatechin, procyanidins B_1 and B_2 and trans-5-O-ceffeoyl-D-quinic acid (Tremocoldi *et al*., 2018).

1.3.7 STRUCTURES OF COMPOUNDS IDENTIFIED AND ISOLATED COMPOUNDS

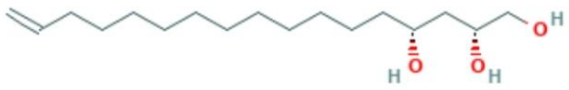


Fig 1.3.7a Structure of 1,2,4-trihydroxyheptadec-16-ene

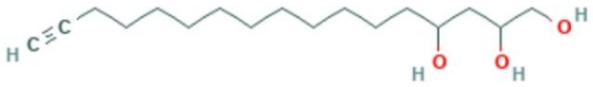


Fig 1.3.7b Structure of 1,2,4-trihydroxyheptadec-16-yne

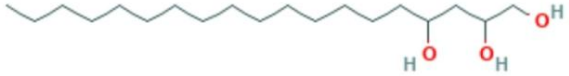


Fig 1.3.7c Structure of 1,2,4-trihydroxynonadecane

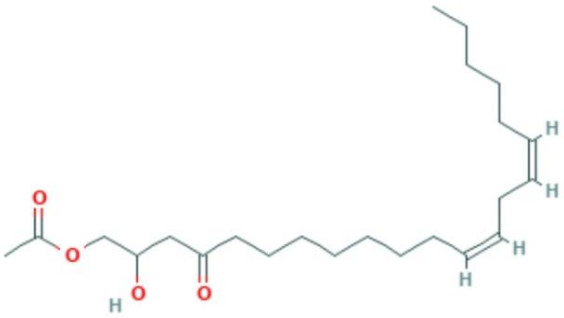


Fig 1.3.7d Structure of persin

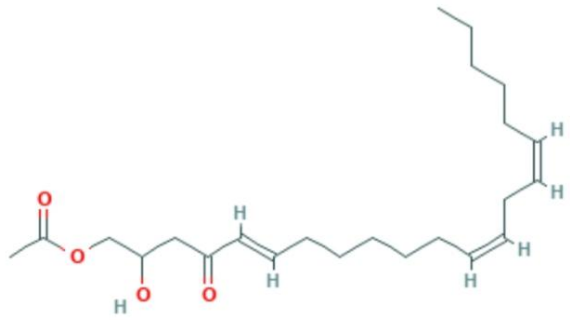


Fig 1.3.7e Structure of Persenones A

Fig 1.3.7f Structure of Persenones B

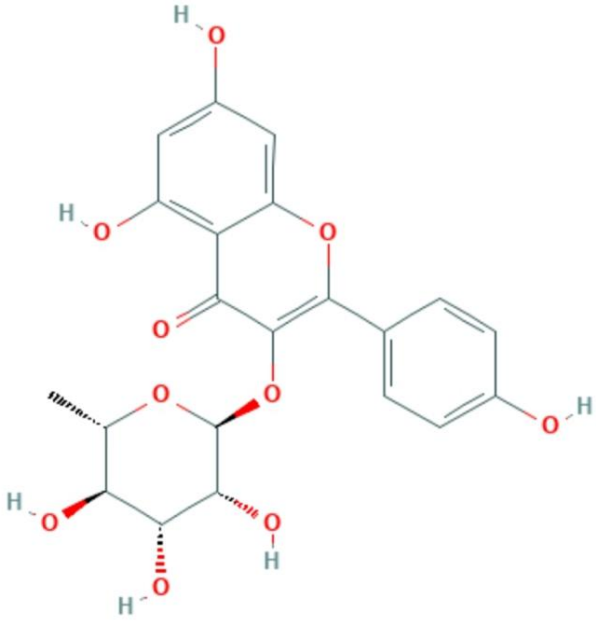


Fig 1.3.7g. Structure of Afzelin

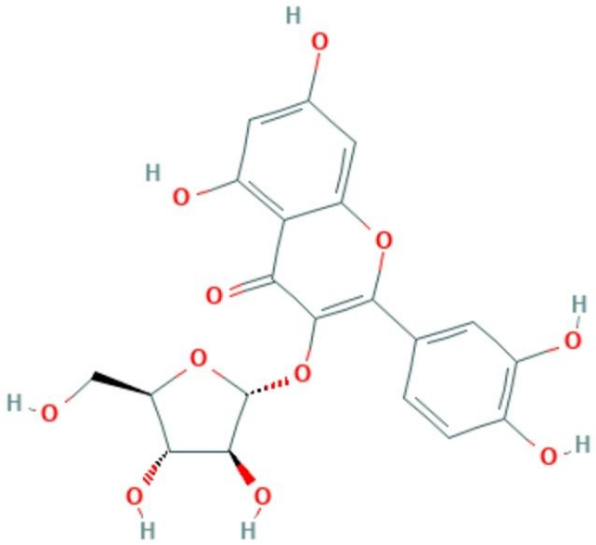


Fig 1.3.7h. Structure of quercetin 3-O-d-arabinopyranoside

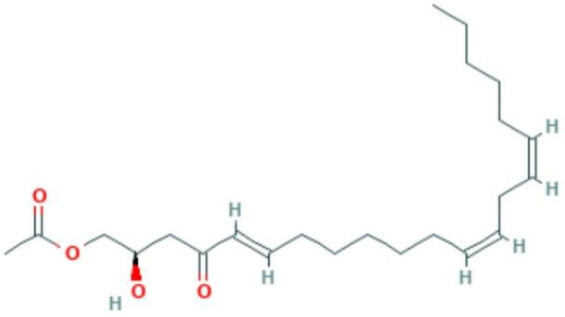


Fig 1.3.7i. Structure of 1-acetoxy-2-hydroxy-4-oxo-heneicosa-5,12,15-triene

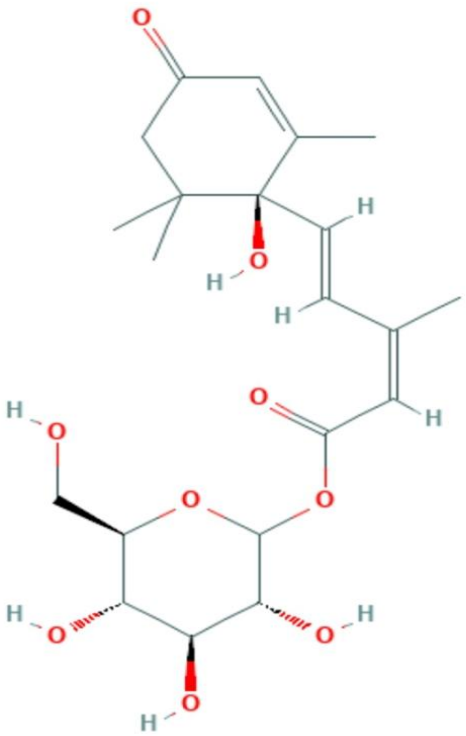


Fig 1.3.7j Structure of (1S,6R)-8-hydroxy abscisic acid-d-glucoside

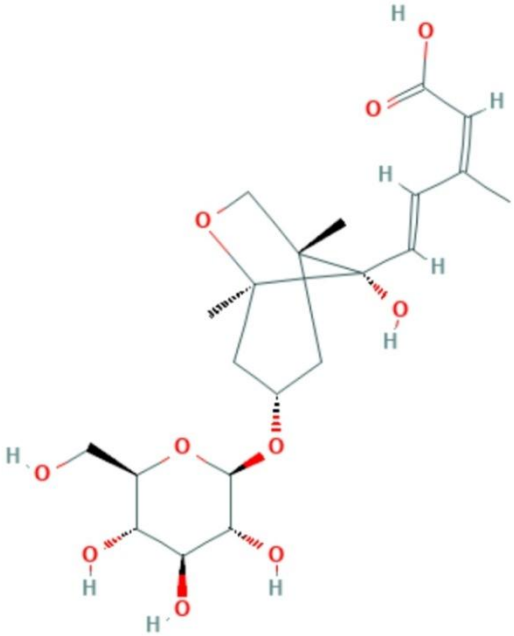


Fig 1.3.7k Structure of (1R,3R,5R,8S)-pi-dihydrophaseic acid-d-glucoside

1.4 AIMS AND OBJECTIVES

The aim of the study is to evaluate the effect of the ethanol seed extract o*f P. americana* on in-vivo antioxidant status of Benign prostate hyperplasia rats and chemical profiling of its phytoconstituent.

The objective;

1. To determine the effect of ethanolic extract of *Persea americana* seed on the oxidative and lipid preoxidation status of Benign prostate hyperplasia rat.

2. To identify the secondary metabolites present in the ethanolic extract of *P. americana* seed using HPLC-MS.

CHAPTER 2

2.0 MATERIAL AND METHOD

Materials used in the laboratory are as follows:

2.1 SOLVENTS/REAGENTS/DRUGS

Ethanol, chloroform, hydrogen peroxide (H_2O_2), potassium permanganate (KMnO4), sulphuric acid (H_2SO_4), buffered pyrogallol (0.05 M pyrogallol in 0.1 M phosphate buffer (pH 7.0) trichloroacetic acid (TCA), Eliman's reagent (DTNB), thiobarbituric acid (TBA), acetonitrile, methanol, 0.1% formic acid, Finasteride, Testosteron dacanoate and olive oil

EQUIPMENT

Soxhlet apparatus, Crucible, Water bath, Weighing balance, Separation funnel, Cotton wool, Sample bottles, Scissors, Microplate reader and Spectrophotometer

2.2 METHODOLOGY

2.2.1 PLANT COLLECTION AND PREPARATION

The fresh and ripe fruits of *Persea american* were purchased from from New Benin market, Benin City, Edo state. Identification and authentication was carried out at the University of Benin Herbarium, plant herbarium sample was allocated the herbarium number UBH-P408 and a voucher specimen was curated.

The seed was separated from the "flesh" of the fruit, cut into small pieces and dried under shade for 7 days. Shade dried seed was further dried in the oven at 40^0C for 30 minutes and milled to fine powder. A weighed amount (57 g) of the powder was extracted in a Soxhlet apparatus with 4500 ml of ethanol for 12 hours. Extract obtained was concentrated over a water bath and; dried extract was preserved in a sample bottle at 4^0C till needed.

2.3 ANIMALS

Thirty male albino rats weighing 170-364 g were obtained from the animal house of the Department of Biochemistry, University of Benin and kept in the animal house of the Department of Pharmacology, Faculty of Pharmacy, University of Benin. The animals were maintained under standard conditions humidity, temperature (23-25°C) and natural light 12h light/dark cycle. They were kept in ventilated cages, were given clean drinking water and fed palletized animal feed. All the animals were acclimatized for two weeks prior to the experiment. Ethical approval was obtained from the Faculty of Pharmacy Ethical Review Committee, for the study.

2.4 EXPERIMENT

Animals were divided into six groups (1-6), of five rats in each group. All the animals in the group were induced with benign prostate hyperplasia through daily subcutaneous administration of 3 mg/kg of testosterone decanoate in olive oil, except for group six animals which was the normal control.

Groups one, two, and three, were orally administrated with 100, 200, 400 mg/kg body weight of the ethanol extract of *Persea americana* seed respectively. Group four animals were orally administrated with 4 mg/kg/d of finastride and, this group served as the positive control, while

group five animals were not treated as they served as the negative control. Group six animals (normal control) received only water. Weight of animals was taken weekly throughout the period of the experiment.

After 28 days of administration, the animals were fasted overnight, anaesthetized in a chloroform saturated chamber, sacrificed and blood samples were collected through the inferior venacava into plain bottles.

Blood samples collected were allowed to clot, retract and centrifuged at 3000 rpm for 10 minutes and separated by means of a Pasteur pipette. The plasma was used to assay for serum antioxidant level of Superoxide dismutase (SOD) catalase (CAT) Gluthathione reductase (GR), Gluthathione peroxidase (GPX) activities and Malonidialdhydeconcentration.

2.5 EVALUATION OF SERUM OXIDENT LEVEL

2.5.1 EVALUATION OF SERUM LEVEL OF SUPEROXIDE DISMUTASE TEST (SOD)

The level of SOD in serum was evaluated by the method of Misra and Fridovich (1972) with a slight modification

$$2O_2 + 2H \xrightarrow{SOD} H_2O_2 + O_2$$

Adrenaline auto-oxidizes rapidly in aqueous solution to adnenochrome, whoes concentration can be evaluated at 430 nm using a spectrophotometer. The auto-oxidation of adrenaline depends on the presence of superoxide anions. The enzyme SOD inhibits the auto-oxidation of adrenaline by catalyzing the breakdown of superoxide anions. The degree of inhibition is therefore the activity of SOD and is determined at one unit of the enzyme activity.

The formula for the calculation is

% inhibition = ($O.D_{ref}$ - $O.D_{test}$ × 100)/ $O.D_{ref.}$

However, 1unit of SOD activity is taken as the amount of SOD required to cause 50% inhibition of the auto-oxidation of adrenaline to adrenochrome per minute.

Therefore, unit/mg wet tissue = (% inhibition/50 × S)

Where, S = (mg tissue / volume)

2.5.2 EVALUATION OF SERUM LEVEL OF CATALASE

This evaluation was carried out according to the method of Cohen *et al*, (1970). The method involve the use of spectrophotometer to measure the absorbance of each sample at the wavelength of 535 nm. Blank was prepared containing 5.0 ml of 30 mM H_2O_2, 0.5 ml of distilled water 1.0 ml of 6 M H_2SO_4, 7.0 ml of contained 5.0 ml of 30 mM H_2O_2, 0 ml of distilled water 1.0 ml of 6M H_2SO_4, 7.0ml of 0.01M $KMnO_4$, and 0.5 ml of sample.

The activity of the catalase in each sample was calculated by applying the formula:

Unit/g tissue ={[(ΔOD/min) × Vi × 1000] / [M × V × L × Y]}

Where:

OD = Absorbance of sample test at 535nm

V = Total volume of the reaction mixture = 13.5 ml

M = Molar extinction coefficient of H_2O_2 = 40 m^{-1} cm^{-1}

L = Light path = 1 cm

Vi = Volume of sample serum used = 0.5 ml

Y = mg tissues used.

1 unit of enzyme activity = 1 mole of H_2O_2 consumed per minute

2.5.3 EVALUATION OF SERUM LEVEL OF GLUTATHIONE PEROXIDASE

The reaction mixture consisted of 10 ml of buffered pyrogallol (0.05 M pyrogallol in 0.1 M phosphate buffer (pH 7.0) and 5 ml of 1% hydrogen peroxide. To this was added 0.5ml of the serum and absorbance change was measured at 430 nm wavelength at every 30 seconds for a minute using spectrophotometer.

The principle of the procedure is;

$$2 \text{ pyrogallol} + 3H_2O_2 \xrightarrow{\text{peroxidase}} \text{Purpurogallen} + 5H_2O + CO_2$$

The spectrophotometric appearance of purpurogallen was measured at 430 nm.

The peroxidase activity was calculated using an extinction coefficient of oxidized pyrogallol of $12m^{-1}cm^{-1}$.

The formula used is as follows:

$$\text{Unit/mg} = \{[(\Delta OD_{430nm}/30sec) \times Vi \times Df]/[M \times V \times L \times S]\}$$

Where;

OD = Absorbance of sample test at 430 nm

V = Total volume of the reaction mixture = 15.5 ml

Df = Dilution factor = 1

M = Molar extinction coefficient of purpurogallen = 12 m-1cm-1.

L = Light path = 1 cm

V = volume of sample homogenate used = 0.5 ml.

S = mg of protein in tissue used

The result was expressed in unit/mg protein, where 1unit of enzyme activity is 1mole of pyrogallol oxidized per minute

2.5.4 EVALUATION OF SERUM LEVEL OF GLUTATHIONE REDUCTASE

This is carried out according to the method of Teltze (1969), by the comparison of the absorbance of the blank, the standard and the samples. For the blank, 2ml of trichloroacetic acid (TCA) was measured into a test-tube, same was done for the standard, but for the sample, 0.5ml of plasma was added to 2.0ml of TCA, all the test-tubes were mixed respectively and allowed to stand for 30 minutes at room temperature, thereafter, they were centrifuge at 5000 rmp for 10 minutes. To the blank, 0.5 ml of Eliman's reagent (DTNB), 3.0 ml of phosphate buffer and 0.05ml of distilled water were added respectively and mixed. To the standard, 0.5 ml of Eliman's reagent, 3.0 ml of phosphate buffer and 0.05 ml of the standard glutathione were added respectively and mixed, while for the sample, 0.05 ml of the supernatant was mixed with 0.5 ml of DTNB and 3.0 ml of phosphate buffer. The concentration of the sample was determined with the aid of spectrophotometer of which the absorbance was read at 412 nm wavelength.

The procedure was carried out in triplicate.

The calculation of the concentration of GSH was done using the formula

Concentration of GSH = (absorbance of sample/absorbance of standard) × concentration of standard.

Concentration of standard in M = 0.0002mole/L

Concentration of standard in g/L = mole/L × molar mass of GSH

= 0.0002 mole/L × 307.33

= 0.0614 g/L

2.5.5 EVALUATION OF SERUM LEVEL OF MALONIDIALDHYDE (MDA)

According to Vershaey and Kale (1990), the assay was done by the addition of 0.6ml of the sample containing the MDA with 3.0 ml of thiobarbituric acid (TBA) which formed a MDA-TBA_2 adduct (red color complex) that absorbs light at 535nm with the of spectrophotometer.

The calculation of the MDA concentration of each of the samples were calculated using extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} in the formula:

Units/ml= (OD × Vi ×1000)/(A x V× L × Y)

Where,

OD = Absorbance of sample test at 535 nm

Vi = Total volume of the reaction mixture = 3.60 ml

A = Molar extinction coefficient of product = $1.56x10^5$ M^{-1} cm^{-1}

L = Light path = 1 cm

V = Volume of sample homogenate used = 0.6 ml

Y = mg of tissue used

2.6. HPLC-MSANALYSIS

Sample was dissolved in water and the aqueous sample was used for HPLC-MS in the electrospray ionization mode. High performance liquid chromatography was conducted by employing the Shimadzu LC-MS 2020 equipped with C8-3 column (2.1×150 mm) and two LC-20 ADXR pumps. The device also consist of a photodiode array detector (model LC-2030/2040) and nitrogen was used as the nebulizing and drying gas. The mobile phase of the separation system consist of water (D), acetonitrile (C), methanol (B) and 0.1% formic acid (A). Base peaks were obtained at scan speed of 5000u/sec from 100 (startm/z)–1000 (endm/z) atomic mass unit in the positive $[M+H]^+$ ionization mode.

The spectra generated within the retention time frame of 0.00-50.00 was monitored using the Shimadzu Lab solution software for liquid chromatography mass spectrometry. The result obtained was exported in CDF format and used for the successive steps required for compound identification with mzmine software (version 2.53). The software provides a graphical interface that allows for result filtering and noise minimization to ensure a better peak analysis (Pluskal *et al*., 2020). Prior to identification, five preliminary steps of data analysis was performed viz: mass detection, chromatogram building, peak deconvolution, peak de-isotoping and data filtering following the protocol described by Olasehinde *et al* (2019), with a few modifications. The data obtained following the preliminary steps was then used for identification using the mzmine online search tool which compares the m/z ratio of the peaks to that available on online database

(primarily Pubchem, mass bank and Kegg web server). The compounds were further tentatively characterized by comparing the fragmentation pattern of the top hits compounds from the online servers to that obtained from the HPLC-MS analysis.

2.7 STATISTICAL ANALYSIS

Results are presented as means +/- standard error of mean (SEM). Statistical analysis was carried out using one way analysis of variance (ANOVA), followed by Dunnelt's post NOC test to assess significant differences among different group. All analysis were performed using graph-pad prism instat version 7.0 of GraphPad Software Inc. in California, USA.

CHAPTER 3

RESULTS

3.1 PERCENTAGE YIELD

The percentage of extract obtained from the dried seed of *P. americana* is 1.87%, after extraction with ethanol.

3.2 EFFECT OF ETHANOL SEED EXTRACT OF *P. americana* ON SUPEROXIDE DISMUTASES

The extract at the dose of 100 mg/kg was observed to significantly ($p< 0.0001$) increase the serum concentration of SOD. This increase was higher than that seen with the 200 and 400 mg/kg dose of the extract respectively.

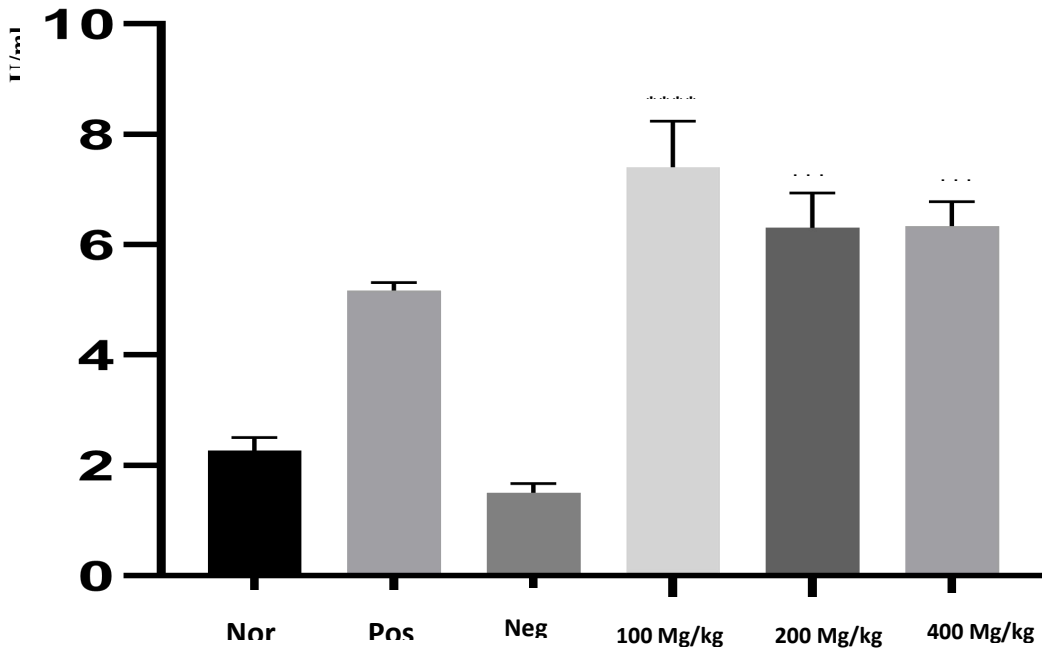


Figure 3.1 :- Effect of ethanol seed extract of *P. americana* on serum concentration of SOD

Results are represented as mean± standard error of mean (SEM) of each groups.

- * = P ≤ 0.05
- ** = p ≤ 0.01
- *** = p ≤ 0.001
- **** = p ≤ 0.0001

3.3 EFFECT OF ETHANOL SEED EXTRACT OF *P. americana* on CATALASE

The results for the activity of the ethanolic extract of *P. americana* seed on the oxidative enzymes, catalase in benign prostatic hyperplasia rat are presented in figure 3.2. It was observed that the animals treated with 100 mg/kg of the extract had the highest concentration of catalase followed by 400 mg/kg.

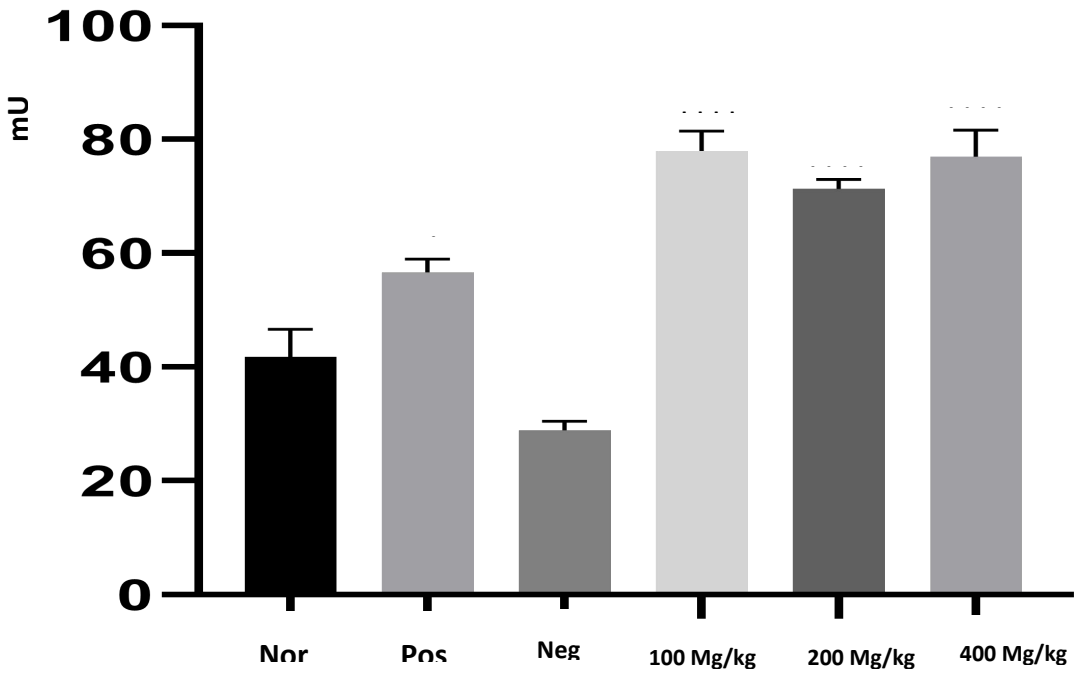


Figure 3.2:- Effect of ethanol seed extract of *P. americana* on serum concentration of catalase

Results are represented as mean± standard error of mean (SEM) of each groups.

- * = $P \leq 0.05$
- ** = $p \leq 0.01$
- *** = $p \leq 0.001$
- **** = $p \leq 0.0001$

3.4 EFFECT OF ETHANOL SEED EXTRACT OF *P.americana* ON GLUTATHIONE PEROXIDASE

The results for the activity of the ethanolic extract of *P. americana* seed on glutathione peroxidase enzyme in benign prostatic hyperplasia rat are presented in figure 3.3. The extract linearly (dose - dependent) and significantly increased the serum concentration of glutathione peroxidase indicating up - regulation of the enzyme activity. It was discovered that animals treated with 400 mg/kg of the extract produced the highest concentration of glutathione peroxidase, followed by 200 mg/kg.

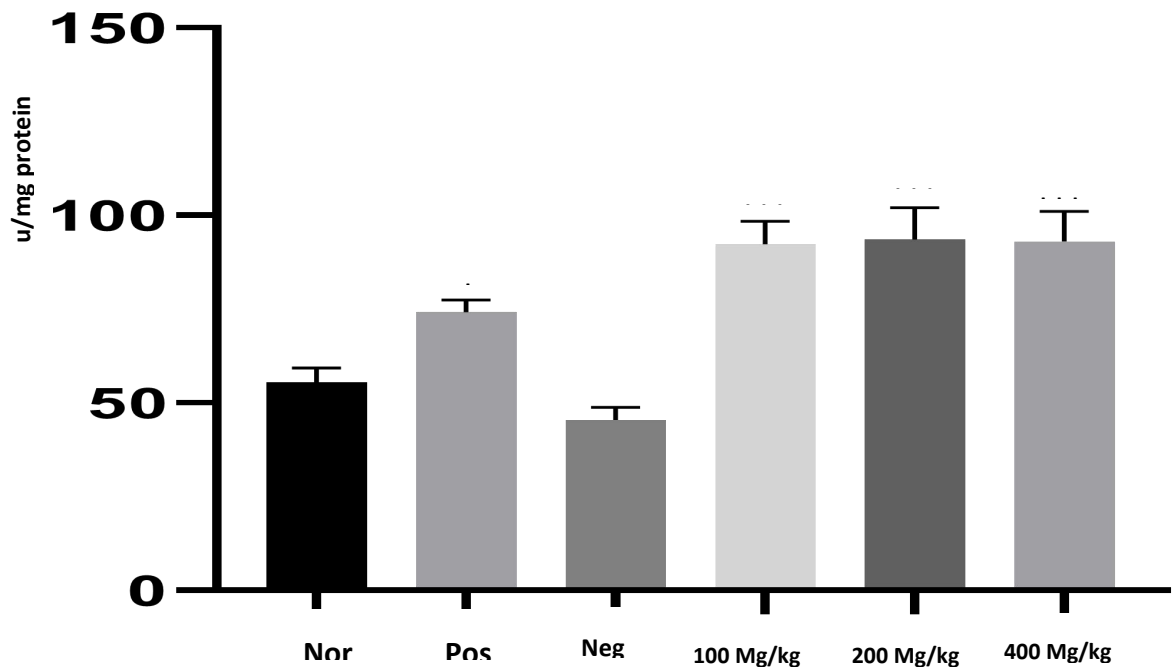


Figure 3.3:- Effect of ethanol seed extract of *P. americana* serum GPx testosterone induced BPH rat

Results are represented as mean± standard error of mean (SEM) of each groups.

- * = $P \leq 0.05$
- ** = $p \leq 0.01$
- *** = $p \leq 0.001$
- **** = $p \leq 0.0001$

3.5 EFFECT OF ETHANOL SEED EXTRACT OF *P.americana* ON GLUTATHIONE REDUCTASE

The results for the activity of the ethanolic extract of *P. americana* seed on oxidative enzyme glutathione reductase in benign prostatic hyperplasia rat are presented in figure 3.4. It was observed that 400mg/kg produced the highest concentration of the enzyme followed by 100mg/kg of the extract.

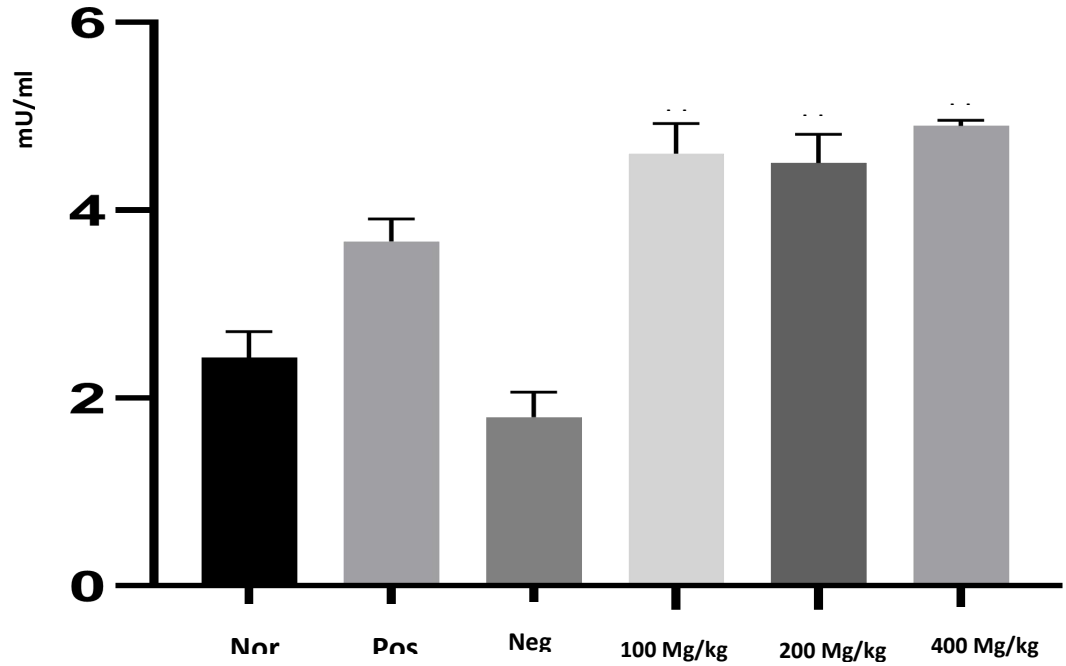


Figure 3.4:- Effect of ethanol seed extract of *P. americana* on GR

Results are represented as mean± standard error of mean (SEM) of each groups.

- * = P ≤ 0.05
- ** = p ≤ 0.01
- *** = p ≤ 0.001
- **** = p ≤ 0.0001

3.6 EFFECT OF ETHANOL SEED EXTRACT OF *P. americana* ON MALONIDIALDHYDE

The results for the activity of ethanolic extract of *P. americana* seed on malonidialdhyde in benign prostatic hyperplasia rat are presented in figure 3.5. MDA is a serrogate of lipid preoxidation, as a result decrease in MDA is indicative of reduction in lipid preoxidation. 400 mg/kg significantly ($p< 0.0001$) decreased the serum concentration of MDA. This decrease was lower than that seen with the 200 and 400 mg/kg dose of the extract respectively.

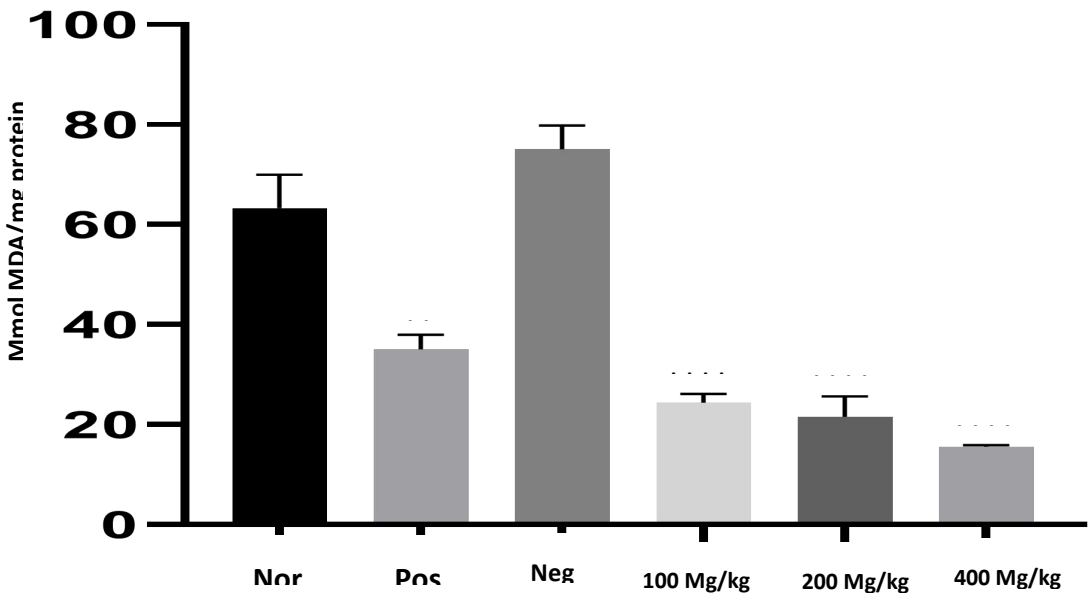


Figure 3.5:- Effect of ethanol seed extract of *P. americana* on MDA

Results are represented as mean± standard error of mean (SEM) of each groups.

- * = $P \leq 0.05$
- ** = $p \leq 0.01$
- *** = $p \leq 0.001$
- **** = $p \leq 0.0001$

3.7 RESULT OF HPLC-MS ANALYSIS Of THE ETHANOL SEED EXTRACT OF *P. americana*

Eleven compounds were tentatively identified in the ethanol seed extract of *P. americana* using HPLC - spectrometry. The identities and characteristics of the compounds are presented in table 3.1. Equally the chromatogram of the compounds is presented in figure 3.6

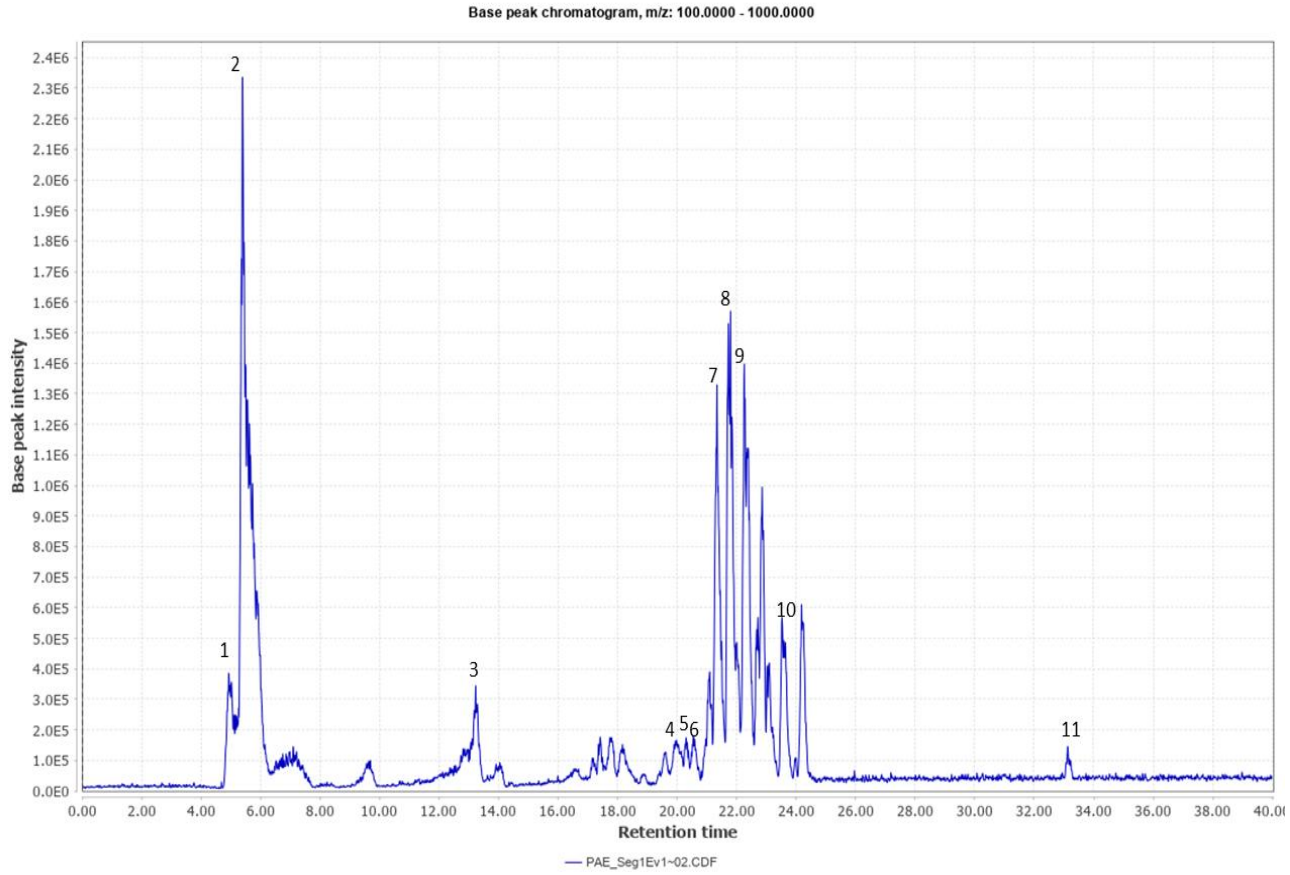


Figure3.7:- Total ion concentration of ethanol seed extract of *P. americana* showing identified metabolites and detected peaks of unidentified metabolites. The identities, molecular formula, molecular weight, mass to charge ratio (m/z) and retention time in minutes (RT) are indicated in table 1.

Table2: Compounds tentatively identified from *Persea americana* by LCMS

Peak#	Name	Molecular formula	Molecular weight(g/mol)	Ret.Time (min)	m/z
1	2-Cyanopyridine	$C_6H_4N_2$	104,11	4,97	104,30
2	3',6-Dimethylflavone	$C_{17}H_{14}O_2$	250,29	5,41	251,05
3	3-O-Acetyldiosgenin	$C_{29}H_{44}O_4$	456,67	13,24	455,10
4	Thiamine mononitrate	$C_{12}H_{17}N_5O_4S$	327,32	19,98	325,20
5	Abscisic acid	$C_{15}H_{20}O_4$	264,32	20,31	262,25
6	3',4',5,7-Tetramethyl quercetin	$C_{19}H_{18}O_7$	358,11	20,57	359,20
7	Taxifolin	$C_{15}H_{12}O_7$	304,25	21,34	305,25
8	Epigallocatechin	$C_{15}H_{14}O_7$	458,37	21,76	307,20
9	Dopaxanthin	$C_{18}H_{18}N_2O_8$	390,30	22,86	391,20
10	4-methylumbelliferyl glucuronide	$C_{16}H_{16}O_9$	352,29	23,57	351,20
11	Digoxin	$C_{41}H_{64}O_{14}$	780,94	33,14	780,50

CHEMICAL STRUCTURES OF THE IDENTIFIED COMPOUNDS

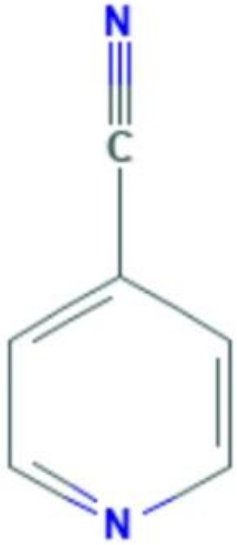
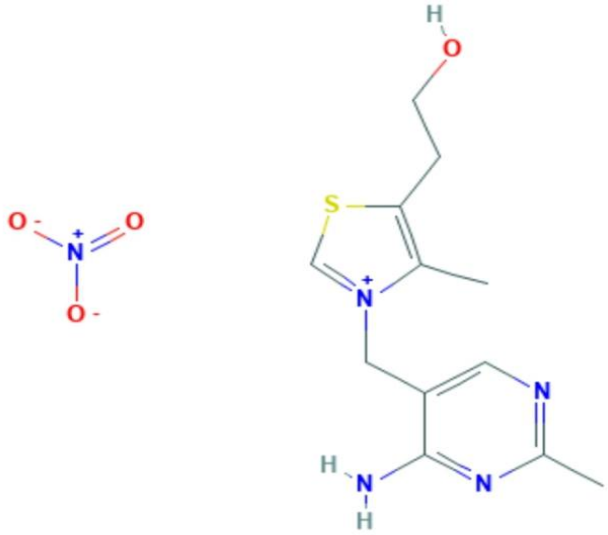


Fig. 3.7a Structure of cyanopyridine



The chemical structure of thiamine mononitrate

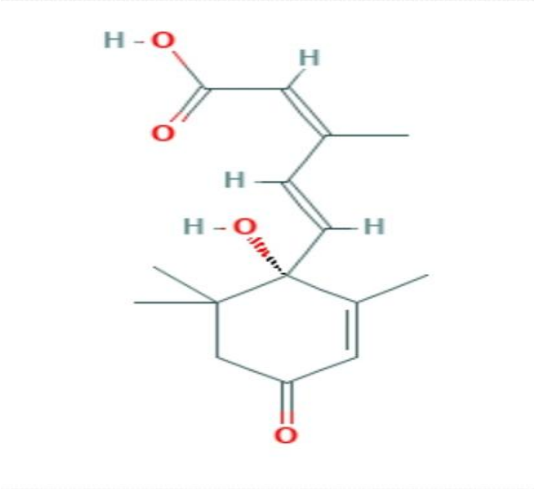


Fig. 3.7b Structure of Abscisic acid

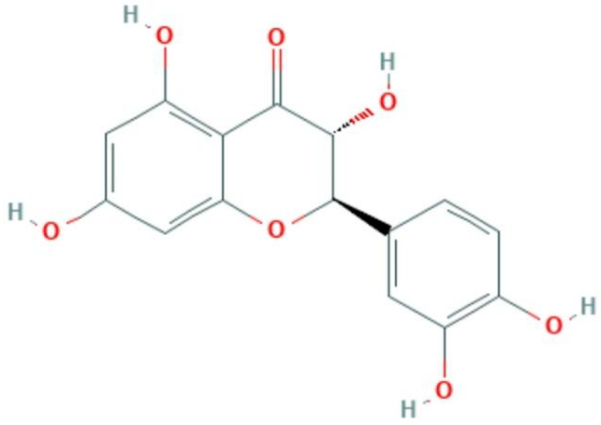


Fig. 3.7c Structure of taxifolin

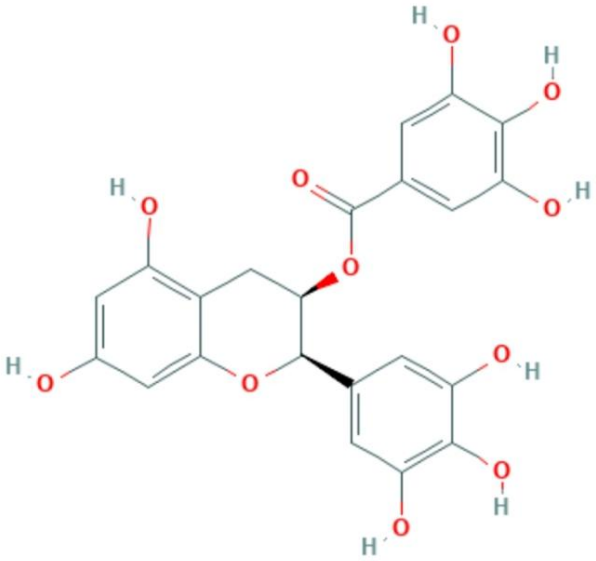


Fig. 3.7d Structure of Epigallocatechin gallate

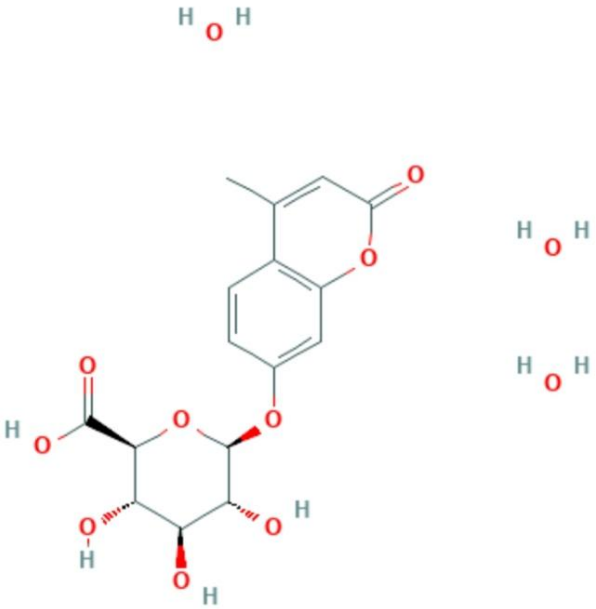


Fig. 3.7e Structure of 4- methylumbrelliferyl glucuronide.

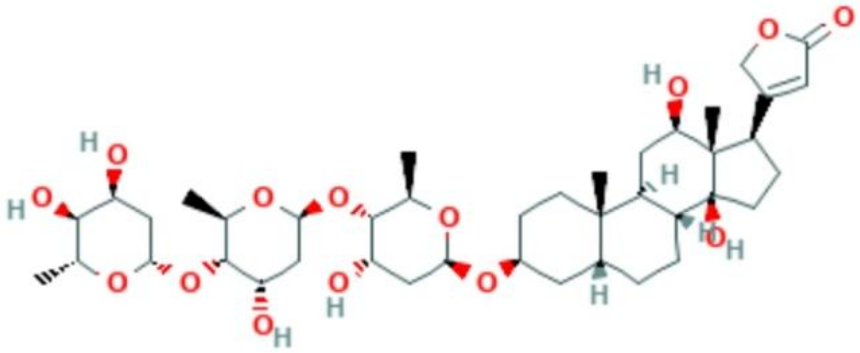


Fig. 3.7f Structure of Digoxin

CHAPTER 4

DISCUSSION

This study was aimed at evaluating the effect of the ethanol extract of *P. americana* seed on in-vivo antioxidant and lipid preoxidation status of benign prostatic hyperplasia rat and chemical profiling of its phytochemical constituents.

BPH is an age-related disease associated with hormonal changes, increased proliferation and suppression of apoptosis of prostatic cells (Novara *et al*., 2006; Liu *et al.,* 2007). The development of BPH is associated with cellular damage induced by oxidative stress (Aydin *et al*., 2006; Mchedlidze and Shioshvili, 2006).

The extent of reactive oxygen species-induced oxidative damage can be exacerbated by a decreased efficiency of antioxidant defense mechanisms. Balance between oxidative stress (OS) and the antioxidant component of the cells has also a role in developing prostate disease (Khandrika *et al*., 2009).

From the results of antioxidant and lipid preoxidation status using level of antioxidant enzymes; superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and malonidialdhyde as the basis of the antioxidant status in benign prostatic hyperplasia rat.

The superoxide dismutase SODs convert superoxide radical into hydrogen peroxide and molecular oxygen. In the present study, ethanol seed extract of *P. americana* at the dose of 100 mg/kg was observed to significantly ($p< 0.0001$) increase the serum level of SOD compared to the negative control group. Although, 200 and 400 mg/kg dose equally produce significant increase in SOD level; the effect observed with the 100 mg/kg was seen to be higher.

Catalase is a common enzyme found in almost all living organisms which are exposed to oxygen, where it function to catalyze the decomposition of hydrogen peroxide to water and oxygen (Cohen *et al.*, 1970). From the results obtained in this study, the extract at lower dose of 100 mg/kg significantly ($p< 0.0001$) increased the serum level of the catalase enzyme. This increase was higher than that observed with the 200 and 400 mg/kg doses respectively.

The biochemical function of glutathione peroxidase (GPX) is to reduce lipid hydroperoxides to their corresponding alcohols and reduce free hydrogenperoxide to water (Flohe and Gunzler,1984). Lower activities of SOD (Arsova-Sarafinovska *et al*., 2009; Aydin *et al*., 2006), GPX (Arsova-Sarafinovska *et al*., 2009; Merendino *et al*., 2003) versus corresponding controls have been shown in BPH patients; however, one of these studies (Arsova-Sarafinovska *et al*., 2009) reported different results between patients from Macedonia and Turkey, the latter showing a decreased activity only in GPX, indicating the importance of GPX as measure of antioxidant activity in BPH. The extract however, dose-dependently, increased the activity of GPX significantly ($p< 0.01$) in the treated animals compared to the control.

The significance of glutathione reductase is to give an idea of reduced glutathione (Teitze, 1969). Glutathione plays a key role in maintaining intracellular antioxidant defenses by scavenging reactive oxygen species and regenerating other antioxidant molecules. In the cytoplasm, reduced glutathione may dissociate hydrogen peroxides (H_2O_2) resulting in increased oxidized glutathione which, subsequently, may be recycled to glutathione by glutathione reductase through the nicotinamide adenine dinucleotide phosphate (NADPH)-dependent redox pathway thereby sustaining adequate levels of glutathione (Shukla *et al*., 2020). Although, glutathione peroxidase is the primary antioxidant enzymes, glutathione reductase helps to increase the level of glutathion. The ethanolic extract of *P. americana* showed significant increase in the level of

glutathion reductase, at the different treatment doses ($p<0.001$), compared to the negative control group animals. The observed increase was higher than that produced by the reference drug (finastride).

Malondialdehyde which is formed the breakdown of polyunsaturated fatty acids serves as a convenient marker for the determination of the extent of lipid peroxidation (Vershney and Kale,1990). Free radicals cause attack on polyunsaturated membrane lipid (lipid peroxidation) generating a product called malondialdehyde (MDA) (De Zwart *et al*., 1999). Serum levels of malondialdehyde however may be elevated in any of the prostatic lesions (Mittal and Scrivastava, 2005). Free radicals are unstable in nature; they appear transiently in the system, thus their assay is difficult. However, before its disappearance, it ensures some degree of damage to cellular organelles. Its tendency to attack membrane polyunsaturated lipid leading to its peroxidation remains the evidence of free radical injury. In the course of this reaction, a product known as malondialdehyde is produced and this is used as an index of free radical injury because it is more stable in the system. (Oparinde *et al*., 2013). Therefore, increase in the level of MDA is indicative of high incident of free radical injury, which is included in the pathophysiology of BPH. However, a decrease in the level of MDA is indicative of reduction in free radical injury. This study, elucidated the effect of ethanolic extract of *P. americana* seed on the testosterone induced BPH, and it was observed that, at all the treatment doses, the extract caused a significant reduction in the level of MDA with a p-value of $p<0.0001$, compared with that of finastride of significance $p<0.01$, however, 400mg/kg dose has the lowest value of 15.53 ± 0.38, while the positive control (finastride) has the value of 35.07 ± 2.89.

Identification of some of the secondary metabolites present in the ethanolic extract of *P. americana* seed was carried out using HPLC-MS. Compounds identified in the extract include;

2-Cyanopyridine, 3',6- Dimethylflavone, 3-O-Acetyldiosyenin, Thiamine mononitrate, Abscisic acid, 3',4',5,7- Tetramethylquercetin, Taxifolin, Epigallocatechin, Dopaxanthin, 4-methylumbelliferyl glucuronide and Digoxin.

Cyanopyridine, is a pyridine derivative that has attracted attention as its many derivative have varied pharmacological activities, and are used in the industrial production of nicotinamide, nicotinic acid and isonicotinic acid. Fused cyanopyridines also have wide range of pharmacological activity. Among these compounds, 2-amino-3-cyanopyridine derivatives have been reported to possess antiviral, antibacterial, and fungicidal activities (Ibrahima *et al*., 2006; Prakash *et al*., 1988). 2-Amino-3-cyanopyridine derivatives were also reported as novel IKK-β inhibitors (Murata *et al*., 2003), A2A adenosine receptor antagonists (Mantri *et al*., 2008). However, the antioxidant performance of these derivatives did no have scavenging efficacies of typical antioxidant agents like vitamin C (Nabil *et al*., 2020).

Thiamine mononitrate and thiamine hydrochloride are regarded as effective sources of vitamin B1. Thiamine (vitamin B1) is a water soluble cation vitamin, which was first identified in the vitamin B family. (Lee *et al*., 2013). It is reported to play a significant role in glucose metabolism and used as a co-factor in several enzyme systems including production of reducing substances used in oxidative stress defense (Lee *et al*., 2013; Potegato *et al*., 2019). Invited and in-vivo antioxidant activities have been cited for thiamine (Gliszazynska - Swigto, 2016). Thiamine has been reported to inhibit lipid peroxidation in the rat liver and free radical oxidates of oleic acid in in-vitro. The antioxidant activity is related to it's ability to successfully transfer $2H^-$ from NH_2 group of the pyrimidine ring and H^+ from the thiazale ring to reduced substrate (Lukenka *et al*., 2007).

Abscisic acid (ABA) is a plant hormone, which functions in many plant developmental processes, including seed and bud dormancy, the control of organ size and stomatal closure. It is especially important for plants in the response to environmental stresses, including drought, soil salinity, cold tolerance, freezing tolerance, heat stress and heavy metal ion tolerance (Finkelstein and Ruth, 2013). Abscisic acid (ABA) has shown efficacy in the treatment of diabetes and inflammation; however, its molecular targets and the mechanisms of action underlying its immunomodulatory effects remain unclear. (Bassaganya-Riera *et al*., 2011). Induction of ABA has been shown to led to a relatively slight increase in the levels of $O2^-$ and H_2O_2 and induction of the capacity of whole antioxidant defence systems against oxidative stress (Jiang *et al*., 2001).

Taxifolin (5,7,3',4'-flavan-on-ol), also known as dihydroquercetin, belongs to the subclass flavanonols in the flavonoid group of compounds (Makena *et al*., 2009). Taxifolin shows promising pharmacological activities in the management of inflammation, tumors, microbial infections, oxidative stress, cardiovascular, and liver disorders (Sunil *et al*., 2019). Topal *et al*, (2016), in a study to determine of the antioxidant potential of taxifolin, discovered that, taxifolin demonstrated 81.02% inhibition of linoleic acid emulsion peroxidation in 30 mcg/ml concentration, clearly showing that taxifolin had marked antioxidant, reducing ability, radical scavenging and metal-chelating activities.

Epigallocatechin gallate (EGCG) is a unique plant compound that has attracted a lot of attention for its potential positive impact on health. It's thought to reduce inflammation, aid weight loss, and help prevent heart and brain disease (Hill, 2019). EGCG and other related catechins have been reported to act as potent antioxidants that may protect against cellular damage caused by free radicals (Kim *et al*., 2014). Based upon its chemical structure, EGCG is often classified as an antioxidant. However, direct treatment of cells with EGCG results in production of hydrogen

peroxide and hydroxyl radicals in the presence of Fe (III). Thus, EGCG may functions as a pro-oxidant in some cellular contexts. Recent investigations have revealed many other direct actions of EGCG that are independent from anti-oxidative mechanisms (Kim *et al*., 2014).

4-methylumbelliferyl glucuronide (4-MUG), is a metabolite of 4-Methylumbelliferone (4-MU). It is reported to inhibits hyaluronan (HA) synthesis and is an approved drug used for managing biliary spasm. However, rapid and efficient glucuronidation is thought to limit its utility for systemically inhibiting HA synthesis. In particular, 4-MU in mice has a short half-life, causing most of the drug to be present as the metabolite 4-methylumbelliferyl glucuronide (4-MUG), which makes it remarkable that 4-MU is effective at all. 4-MUG contributes to HA synthesis inhibition. It was observed that oral administration of 4-MUG to mice inhibits HA synthesis, promotes FoxP3+ regulatory T-cell expansion, and prevents autoimmune diabetes. Indeed, 4-MU and 4-MUG were almost equally effective over a range of concentrations at inhibiting HA synthesis by cancer cell lines in vitro. Both were likewise equally effective in treating autoimmunity in a mouse model (Nagy *et al*., 2018).

Digoxin, a cardiac glycoside, has inotropic effects in addition to effects on cardiac output. It is used to treat heart failure and atrial fibrillation and has other off-label uses. Digoxin has been shown to reduce hospitalization rates without affecting mortality rates in patients with heart failure. It is effective for rate control in patients with atrial fibrillation, but its influence on mortality rates is a source of controversy, as its use is limited because the drug has a narrow therapeutic index and close monitoring required with its use. Despite its limitations, however, digoxin has a place in therapy (Chen *et al*., 2015). Off-label uses of digoxin include; fetal tachycardia, supraventricular tachycardia, corpulmonale, and pulmonary hypertension (Micromedx Healthcare series, 2013).

The antioxidant activities of the ethanol seed extract of *P. americana* as demonstrated in this study can be linked to the presence of the identified compounds in the extract, some of which have been cited to have antioxidant effects. However, other compounds in the extract which have not been identified may also play in the observed activity of the extract.

The study provide scientific basis for use of the seed of *P. americana* in ethno medicine to manage BPH. However, further studies are advocated to explore these roles.

CONCLUSION

This present work has shown that the ethanolic extract of *Pesea americana* seed possess antioxidant properties in testosterone induced benign prostatic hyperplasia.

Furthermore, the extract possesses some important phytochemicals which have great pharmacological activities in the treatment and management of some diseases and sicknesses in men. Compounds such digoxin, epigallocatechingallate, taxifolin, abscisicacid, 4-methylumbelliferyl glucuronide, cyanopyridines and Thiamine mononitrate.

REFERENCES

Abe F, Nagafuji S, Okawa M, Kinjo J, Akahane H, Ogura T (2005). Trypanocidal constituents in plants 5. Evaluation of some Mexican plants for their trypanocidal activity and active constituents in the seeds of *Persea americana*. Biological and Pharmaceutical Bulletin. 28:1314–7.

Akerale O, Heywood V and Synge H (1991). Conservation of medicinal plants, Cambridge: Cambridge University press; pp. 1-362.

Al-Ansari A, Younes N, Sampige VP, Al-Rumaihi K, Ghafouri A, Gul T, Shokeir AA (2010). Hamad Medical Corporation, Doha, Qatar.

Andriole G., Bruchovsky N., Chung L. W., Matsumoto A. M., Rittmaster R., Roehrborn C., et al. (2004). Dihydrotestosterone and the prostate: the scientific rationale for 5α-reductase inhibitors in the treatment of benign prostatic hyperplasia. The Journal Urology. 172: 1399–1403.

Anita BS, Okokon JE, and Okon PA (2005). Hypoglycemic activity of aqueous leaf extract of *Persea americana* Mill.. Indian Journal Pharmacology, 37(5), 325-326.

Arsova-Sarafinovska Z, Eken A, Matevska N, Erdem O, Sayal A, Savaser A, Banev S, Petrovski D, Dzikova S, Georgiev V, Sikole A, Ozgök Y, Suturkova L, Dimovski AJ, Aydin A (2009). Increased oxidative/nitrosative stress and decreased antioxidant enzyme activities in prostate cancer. Clinical Biochemistry. 42(12):1228-35. PMID: 19465015.

Asada Y., Sonoda T., Ojiro M., Kurata S., Sato T., Ezaki T., et al. (2001). 5α-reductase type 2 is constitutively expressed in the dermal papilla and connective tissue sheath of the hair follicle in vivo but not during culture in vitro. The Journal of Clinical Endocrinology Metabolism. 86: 2875–2880.

Aydin A, Arsova-Sarafinovska Z, Sayal A, Eken A, Erdem O, Erten K, Ozgök Y, Dimovski A (2006). Oxidative stress and antioxidant status in non-metastatic prostate cancer and benign prostatic hyperplasia. Clinical Biochemistry. 39(2):176-9.

Barbalat Y, Velez CM, Sayegh IS, Chung ED (2016). Therapeutic advances in urology 8 (3), 181-191.

Bartsch G, Rittmaster RS, Klocker H (A2002). "Dihydrotestosterone and the concept of 5alpha-reductase inhibition in human benign prostatic hyperplasia". World Journal of Urology. 19 (6): 413–25.

Bassaganya-Riera J, Guri JA, Lu P, Climent M, Carbo A, Sobral BW. Horne WT, Lewis SN, Bevan DR, and Hontecillas R (2011). Journal of Biological Chemistry. 286(4): 2504–2516.

Black L, Naslund MJ, Gilbert TD, Davis EA, Ollendorf DA (2006). "An examination of treatment patterns and costs of care among patients with benign prostatic hyperplasia". The American Journal of Managed Care. 12 (4 Suppl): S99–S110.

Blankstein U, Van Asseldonk B, Elterman DS (2016). "BPH update: medical versus interventional management". The Canadian Journal of Urology. 23 (Suppl 1): 10–5.

Braquet P and Hosford D (1991). Pharmacology and the development of natural PAF antagonist as therapeutic agents, Journal of Ethnopharmacology pp. 32: 135-139

Castillo-Juarez I, Gonzalez V, Jaime-Aguilar H, Martinez G, Linares E, Romero I (2009). Anti-Helicobacteria pylori activity of plants used in Mexican traditional medicine for gastrointestinal disorders. Journal of Ethnopharmacology. 122(2):402–405.

Chen Y, Cai X, Huang W, Wu Y, Huang Y, and Hu Y (2015). Increased All-Cause Mortality Associated With Digoxin Therapy in Patients With Atrial Fibrillation. Medicine (Baltimore). 94(52):

Chen, H; Morrell, PL; Ashworth, V; de la Cruz, M; Clegg, MT (2008). "Tracing the Geographic Origins of Major Avocado Cultivars". Journal of Heredity. 100 (1): 56–65. doi:10.1093/jhered/esn068. PMID 18779226.

Christophersen C, Larsen C and Dimayuga RE (1991). Traditional medicine. A potential resource exploitation of Natural Products. The H.C, Ørsted institute, Copenhagen. pp. 8-12.

Christophersen C, Larsen C, and Dimayuga, RE (1991). Traditional medicine. A potential resource exploitation of Natural products. The H.C, Ørsted institute, Copenhagen. pp8-12 Crown agent London.

Chyou PH, Nomura AM, Stemmermann GN, Hankin JH (1993). "A prospective study of alcohol, diet, and other lifestyle factors in relation to obstructive uropathy". The Prostate. 22 (3): 253–64.

Clark R. V., Hermann D. J., Cunningham G. R., Wilson T. H., Morrill B. B., Hobbs S. (2004). Marked suppression of dihydrotestosterone in men with benign prostatic hyperplasia by dutasteride, a dual 5α-reductase inhibitor. The Journal of Clinical Endocrinology and Metabolism. 89: 2179–2184.

Clement K M, Nanda J, Chapman EK , and Habib FK. Prostate Research Group, Endocrinology Unit, Clinical Biochemistry, University of Edinburgh Cancer Research Centre, Western General Hospital, 4th floor MRC Human Genetics Building, Crewe Road South, Edinburgh EH4 2XU, UK. DOI: https://doi.org/10.1677/JOE-07-0470 Volume 197: Issue 3 Pg 483–491.

Cohen G, Dembiec D, Marcus J (1970). Measurement of catalase activity in tissue extracts. Analytical Biochemistry. 34:30-8.

Cox PA and Balick JM (1994). Ethnobotanical approach to drug discovery. Scientific American pp. 8:83-87

Dalziel JM (1937). The useful plants of west tropical Africa. Crown agent London.

De Almeida, M. Miranda, I. Simoni, M. Wigg, M. Lagrota, S. Costa Flavonol monoglycosides isolated from the antiviral fractions of Persea americana (Lauraceae) leaf infusion Phytother. Res., 12 (1998), pp. 562-567

De Jong Y, Pinckaers JH, Ten Brinck RM, Lycklama à Nijeholt AA. (2014). "Influence of voiding posture on urodynamic parameters in men: a literature review". Nederlands Tijdschrift voor urologie.

del Refugio-Ramos M, Jerz G, Villanueva S, Lopez-Dellamary F, Waibel R, Winterhalter P (2004). Two glucosylated abscisic acid derivates from avocado seeds (*Persea americana* Mill.Lauraceae cv.Hass) Phytochemistry. 65:955–62.

del-Refugio M, Jerza G, Villanueva S, López-Dellamary F, Waibel R Winterhalter P. (2003) https://doi.org/10.1016/j.phytochem.2003.12.007.

Ding H, Chin YW, Kinghorn AD, D'Ambrosio SM (2007). Chemopreventive characteristics of avocado fruit. Seminar in Cancer Biology Journal. 17(5):386-94.

Domergue F, Helms GL, Prusky D, Browse J (2000). Antifungal compounds from idioblast cells isolated from avocado fruits. Phytochemistry. 54:183–9.

Ekor M, Adepoju GKA and Epoyun AA (2006). Protective effect of the methanolic leaf extract of *Persea americana* (avocado) against paracetamol-induced acute hepatotoxicity in rats. International Journal of Pharmacology 2(4): 416-420.

Ellis WJ, Brawer MK (1993). PSA in benign prostatic hyperplasia and prostatic intraepithelial neoplasia. Urology Clinics of North America. 20(4):621-5. PMID: 7505969.

Falodun A, Imieje V, Erharuyi O, Ahomafor J, Jacob MR, Khan S and Hamann MT (2014) http://dx.doi.org/10.4314/ajtcam.v11i4.22.

Feldman BJ, Feldman D (2001). Nature Reviews Cancer 1 (1): 34-45.

Gacci M, Ficarra V, Sebastianelli A, Corona G, Serni S, Shariat SF (2014). "Impact of medical treatments for male lower urinary tract symptoms due to benign prostatic hyperplasia on ejaculatory function: a systematic review and meta-analysis". The Journal of Sexual Medicine. 11 (6): 1554–66.

Gormley GJ, Stoner E, Bruskewitz RC, Imperato-McGinley J, Walsh PC, McConnell JD (1992). "The effect of finasteride in men with benign prostatic hyperplasia. The Finasteride Study Group". The New England Journal of Medicine. 327 (17): 1185–91.

Greco KA, McVary KT (2008). "The role of combination medical therapy in benign prostatic hyperplasia". International Journal of Impotence Research. 20 Suppl 3: S33-43.

Gu F (1997). "Changes in the prevalence of benign prostatic hyperplasia in China". Chinese Medical Journal. 110 (3): 163–6.

Heber D (2002). "Prostate enlargement: the canary in the coal mine?". The American Journal of Clinical Nutrition. 75 (4): 605–6.

Hwang EC, Gandhi S, Jung JH, Imamura M, Kim MH, Pang R, Dahm P (2018). "Naftopidil for the treatment of lower urinary tract symptoms compatible with benign prostatic hyperplasia". The Cochrane Database of Systematic Reviews. 10: CD007360.

Hwang EC, Gandhi S, Jung JH, Imamura M, Kim MH, Pang R, Dahm P (2018). "Naftopidil for the treatment of lower urinary tract symptoms compatible with benign prostatic hyperplasia". The Cochrane Database of Systematic Reviews. 10: CD007360.

Idris, S., Ndukwe, G.I. and Gimba, C.E. (2009). Preliminary phytochemical screening and antimicrobial activity of seed extracts of *Persea Americana* (Avocado Pear). *Bayero Journal of pure and Applied sciences*, 2(1),173 – 176.

Izumi K, Mizokami A, Lin WJ, Lai KP, Chang C. (2013) receptor roles in the development of benign prostate hyperplasia. American Journal of Pathology. 2013 Jun;182(6):1942-9.

Jiang CJ, Shimono M, Sugano S, Kojima M, Yazawa K, Yoshida R, Inoue H, Hayashi N, Sakakibara H and Takatsuji H, (2010). Acid Interacts Antagonistically with Salicylic Acid Signaling Pathway in Rice *Magnaporthe grisea,* Interactions Molecular Plant Microbe Interactions 23(6):791-8

Jiang M, Zhang J, (2001). Effect of Abscisic Acid on Active Oxygen Species, Antioxidative Defence System and Oxidative Damage in Leaves of Maize Seedlings, Plant and Cell Physiology, Volume 42, Issue 11, Pages 1265–1273.

Kang J, Lee G, Jung Y, Youn D, Lim S, Park J & Um J (2017).The Korean Society of Toxicogenomics and Toxicoproteomics and Springer.

Kang JW, Lee GH, Jung Y, You DH, Lim S, Park J and Um JY (2017). The Korean Society of Toxicogenomics and Toxicoproteomics and Springer. DOI 10.1007/S 13273-018-0023-3

Kaplan SA, McConnell JD, Roehrborn CG, Meehan AG, Lee MW, Noble WR (2006). "Combination therapy with doxazosin and finasteride for benign prostatic hyperplasia in

patients with lower urinary tract symptoms and a baseline total prostate volume of 25 ml or greater". The Journal of Urology. 175 (1): 217–20, discussion 220–1.

Kawagishi H, Fukumoto Y, Hatakeyama M, He P, Arimoto H, Matsuzawa T (2001). Liver injury suppressing compounds from avocado (*Persea americana*) J Agric Food Chem. 2001;49:2215–21.

Keehn, A., Taylor, J. & Lowe, F.C. (2016). Phytotherapy for Benign Prostatic Hyperplasia. Current Urology Report 17, 53.

Kim EH, Larson JA, Andriole GL (2016). "Management of Benign Prostatic Hyperplasia". Annual Review of Medicine (Review). 67: 137–51.

Kim HS, Quon MJ, Kim JA (2014). New insights into the mechanisms of polyphenols beyond antioxidant properties; lessons from the green tea polyphenol, epigallocatechin 3-gallate. Redox Biology. 2:187-95.

Kim HS, Quon MJ, Kim JA. (2014). New insights into the mechanisms of polyphenols beyond antioxidant properties; lessons from the green tea polyphenol, epigallocatechin 3-gallate. Redox Biology Journal. 10;2:187-95.

Kinghorn AD and Balandrin MF (1993). Human medicinal agent from plants American Chemical Society Symposium Series 534: 1-348.

Kuang M, Vu A & Athreya S CardioVascular and Interventional Radiology volume 40, pages655–663 (2017)

Kumar B, Koul S, Khandrika L, Meacham RB, Koul HK (2008). Oxidative stress is inherent in prostate cancer cells and is required for aggressive phenotype. Cancer Research. 68:1777–1785.

Lee, S. , Aranyosi A.J., Wong M.D. (2016). Flexible opto-electronics enabled microfluidics systems with cloud connectivity for point-of-care micronutrient analysis. Biosensors. Bioelectronics. 78: 290–299.

Liu YQ, Wu H, Chen H, Liu YL, He J, Kang HY, Sun ZG, Pan G, Wang Q, Hu JL, Zhou F, Zhou KN, Zheng XM, Ren YL, Chen LM, Wang YH, Zhao ZG, Lin QB, Wu FQ, Zhang X, Guo XP, Cheng XN, Jiang L, Wu CY, Wang HY, Wan JM (2015). A gene cluster encoding lectin receptor kinases confers broad-spectrum and durable insect resistance in rice. Nature Biotechnology. 33(3):301–305.

MacDonald R, Tacklind JW, Rutks I, and Wilt TJ. (2012); 109(12): 1756–1761.

MacDonald R, Wilt TJ (2005). "Alfuzosin for treatment of lower urinary tract symptoms compatible with benign prostatic hyperplasia: a systematic review of efficacy and adverse effects". Urology. 66 (4): 780–8.

Makena PS, Pierce SC, Chung KT, Sinclair SE (2009). Comparative mutagenic effects of structurally similar flavonoids quercetin and taxifolin on tester strains Salmonella typhimurium TA102 and *Escherichia coli* WP-2 uvrA. Environmental and Molecular Mutagenesis. 50(6):451-9.

Mantri M, de Graaf O, Veldhoven JV, Goblyos A, Kunzel JKFD, Mulder-KriegerTK, Link R, de Vries H, Beukers MW, Brussee J, Ijzerman AP (2008). 2-Amino-6-furan-2-yl-4-substituted nicotinonitrile as A2Aadenosine receptor antagonists Journal of Medicinal Chemistry. 51: 4449–4455.

Maron JB, Stevenson GW, Tarkington GL, Yancy WC, (2011). CLINICAL GUIDELINE| VOLUME 142, ISSUE 6, E153-E203.

Matsusaka Y, Kawabata J, Kasai T (2003). Antioxidant constituents in avocado (*Persea americana* Mill.) seeds. Nippon Shoukuhin Kaegaku Kogaku Kaishi. 50:550–2.

Mchedlidze MG, Sikharulidze DV, Shioshvili TI (2006). Efficiency of assessment of retinol, alpha-tocopherol and beta-carotene by the liquid chromatography method. Georgian Medical News. (137):102-6. Russian. PMID: 16980761.

McNicholas TA (2016). Medical Devices (Auckl). 9: 115–123.

Merendino RA, Salvo F, Saija A, Di Pasquale G, Tomaino A, Minciullo PL, Fraccica G, Gangemi S (2003). Malondialdehyde in benign prostate hypertrophy: a useful marker? Mediators of Inflammation. 12(2):127-8.

Misra HP, Fridovich I (1972).The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biological Chemistry. 247(10):3170-5. PMID: 4623845.

Morton JF (1987). Avocado; In: Fruits of Warm Climates. Center for New Crops and Plant Products, Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, IN. pp. 91–102. ISBN 978-0-9610184-1-2.

Murata T, Shimizu K, Manganiello VC, Tagawa T, Okumura K, (2002) Expression and role of phosphodiesterase 3 in human squamous cell carcinoma KB cells. Anticancer Drugs. 13(8), 875-80.

Nabil A. Alhakamy, Ahmad O. Noor, Khaled M. Hosny, Jenny Jeehan Nasr, Moustafa M.G. Fouda, Tawfik A. Khattab and Hatem E. Gaffer, (2020). “Synthesis of New

Cyanopyridine Scaffolds and their Biological Activities", Current Organic Synthesis; 17(7) .

Nagy N, Gurevich I, Kuipers HF, Ruppert S, Marshall LP, Xie JB, Sun W, Malkovskiy AV, Rajadas J, Grandoch M, Fischer WJ, Frymoyer AR, Kaber G, Bollyky LP (2019). 4-Methylumbelliferyl glucuronide contributes to hyaluronan synthesis inhibition. GLYCOBIOLOGY AND EXTRACELLULAR MATRICES| VOLUME 294, ISSUE 19, P7864-7877.

Nandwani D (2014). Sustainable Horticultural Systems: Issues, Technology and Innovation. Springer. pp. 176–. ISBN 978-3-319-06904-3.

Nathaniel Owusu-Boadi N, Saah SA, Mensah JK, Badu M, Addai-Arhinand S and Mensah BM (2015). Vol. 9(36), pp. 933- 939, 25 Article Number: B13A36455396. ISSN 1996-0875.

Nayak BS, Raju SS, Chalapathi Rao AV. Wound healing activity of Persea americana (avocado) fruit: a preclinical study on rats. J Wound Care. 2008 Mar;17(3):123-6. doi: 10.12968/jowc.2008.17.3.28670. PMID: 18376654.

Novaraa G, Galfanoa A, Boscolo R, Vincenzo B, Remigio F, Navarretec V, Artibania W (2006). Inflammation, Apoptosis, and BPH: What is the Evidence? European Urology Supplements Volume 5, Issue 4, Pages 401-409.

Oberlies NH, Rogers LL, Martin JM, McLaughlin JL (1998). Cytotoxic and insecticidal constituents of the unripe fruit of *Persea americana.* Journal Natural Products. 61:781–5.

Oelrichs PB, Ng JC, Seawright AA, Ward A, Schaffeler L, MacLeod JK (1995). Isolation and identification of a compound from avocado (*Persea americana*) leaves which causes necrosis of the acinar epithelium of the lactating mammary gland and the myocardium. Journal of Natural Toxins. 3:344–9.

Ofeimun JO and Fanakayo T (2020). Herbal Treatment of Benign Prostatic Hyperplasia: Findings from an Ethnobotanical Survey of Akinyele Local Government Area, Oyo State Nigeria.Tropical Journal of Natural Product Research. 4(3):98-104.

Ojewola R, Oridota E, Balogun O, Ogundare E, Alabi T, Banjo O, Laoye A, Adetunmbi B, Adebayo B, Oluyombo R. (2017). Knowledge, attitudes and screening practices regarding prostatic diseases among men older than 40 years: a population-based study in Southwest Nigeria. Pan African Medical Journal.

Ojewole JA, Amabeoku GJ (2006). Anticonvulsant effect of *Persea americana* Mill (Lauraceae) (Avocado) leaf aqueous extractin mice. Phytotherapy Research. 20:696–700.

Omodamiro OD, Jimoh MA, Ewa IC, (2016). Hepatoprotective and haemopoeitic activity of ethanol extract of *Persea americana* seed in paracetamol induced toxicity in wistar albino rat. International Journal of Pharmacy and Pharmaceutical Research 5(3):149-165.

Oparinde DP, Atiba SA, Adegun PT and Niran-Atiba TA (2013) Serum, Gamma Glutamyl Transferase and Prostate Specific Antigen as Markers of Cancer of the Prostate. The International Journal of Biotechnology, 2013, vol. 2, issue 3, 59-67.

Owolabi MA, Jaja SI and Coker HA (2005). Vasorelaxant action of aqueous extract of the leaves of *Persea americana* on isolated thoracic rat aorta. Fitoterapia, 76: 567-573.

Page ST, Lin DW, Mostaghel AE, Hess DL, True DL, Amory JK, Nelson SP, Matsumoto AM, Bremner WJ (2006). The Journal of Clinical Endocrinology & Metabolism 91 (10): 3850-3856.

Pattanaik S, Mavuduru RS, Panda A, Mathew JL, Agarwal MM, Hwang EC (2018). "Phosphodiesterase inhibitors for lower urinary tract symptoms consistent with benign prostatic hyperplasia". The Cochrane Database of Systematic Reviews. 11: CD010060.

Pisco J, Bilhim T, Pinheiro LC, Fernandes L, Pereira J, Costa NV, Duarte M, Oliveira AG (2016). Prostate Embolization as an Alternative to Open Surgery in Patients with Large Prostate and Moderate to Severe Lower Urinary Tract Symptoms. Journal Vascular and Interventional Radiology. 27(5):700-8.

Porpiglia F, Fiori C, Bertolo R, Garrou D, Cattaneo G, Daniele Amparore D. (2014). https://doi.org/10.1111/bju.12982

Richard M, Manoj H, Sean PM, Roderick E, Jens M, James L, Timothy T, Wilt L. (2012) https://doi.org/10.1002/14651858.CD004135.pub3

Robert G, Descazeaud A, Allory Y, Vacherot F, de la Taille A. (2009) DOI:https://doi.org/10.1016/j.eursup.2009.11.004.

Roberts OR, Jacobson JD, Rhodes T, Klee G, Leiber M, Jacobsen SJ (2004). The Prostate 61 (2): 124-131.

Robinson J (2008). "Post-micturition dribble in men: causes and treatment". Nursing Standard. 22 (30): 43–6.

Rodriguez-Saona C, Trumble JT (2000). Biologically active aliphatic acetogenins from specialized idioblast oil cells. Current Organic Chemistry. 4:1249–60.

Roehrborn C. (2008). Pathology of benign prostatic hyperplasia. International Journal of Impotence Research 20: S11–S18.

Roehrborn CG, Nuckolls JG, Wei JT, Steers W (2007). "The benign prostatic hyperplasia registry and patient survey: study design, methods and patient baseline characteristics". BJU International. 100 (4): 813–9.

Ruth Finkelstein. (2013) Abscisic Acid Synthesis and Response. The Arabidopsis Book 11:e0166. doi:10.1199/tab.0166.

Santillo VM, Lowe FC (2006). "Treatment of benign prostatic hyperplasia in patients with cardiovascular disease". Drugs & Aging. 23 (10): 795–805.

Sarma AV, Wei JT (2012). "Clinical practice. Benign prostatic hyperplasia and lower urinary tract symptoms". The New England Journal of Medicine. 367 (3): 248–57.

Silva J, Silva CM, Cruz F (2014). "Current medical treatment of lower urinary tract symptoms/BPH: do we have a standard?". Current Opinion in Urology. 24 (1): 21–8.

Silva V, Grande AJ, Peccin MS (2019). "Physical activity for lower urinary tract symptoms secondary to benign prostatic obstruction". The Cochrane Database of Systematic Reviews. 4 (4): CD012044.

Srivastava DS, Mittal RD (2005). Free radical injury and antioxidant status in patients with benign prostate hyperplasia and prostate cancer. Indian Journal of Clinical Biochemistry. 20(2):162-5.

Storey, W. B. (1973). "What kind of fruit is the avocado?". California Avocado Society 1973–74 Yearbook. 57: 70–71.

Sunil C, Xu B (2019). An insight into the health-promoting effects of taxifolin (dihydroquercetin). Phytochemistry. 166:112066.

Tcheghebe TO, Nyamen LD, Ngouafong-Tatong F, Seukep A J (2016). Ethnobotanical uses, phytochemical and pharmacological profiles, and toxicity of *Persea americana* mill.: Pharmacologyonline 3:213-221

Thenmozhi A, Shanmugasundaram C, Rao UM. (2012). Biochemical evaluation of anti-diabetic phytomolecule through bioactivity guided solvent fractionation and subfractionation from hydromethanolic (2: 3) extract of Alligator pear Fruit in streptozotocin induced diabetic rats. Journal of Applied Pharmaceutical Science 2: 61.

Thigpen AE, Silver RI, Guileyardo JM, Casey ML, McConnell JD, Russell DW. (1993). Tissue distribution and ontogeny of steroid 5 alpha-reductase isozyme expression; 92(2):903-10.

Topal F, Nar M, Gocer H, Kalin P, Kocyigit UM, Gülçin İ, Alwasel SH. (2016). Antioxidant activity of taxifolin: an activity-structure relationship. Journal of Enzyme Inhibition and Medicinal Chemistry. 31(4):674-83.

Topal F, Nar M, Gocer H, Kalin P, Kocyigit UM, Gülçin İ, Alwasel SH (2016). Antioxidant activity of taxifolin: an activity-structure relationship. Journal Enzyme Inhibition Medicinal Chemistry. 31(4):674-83.

Truzzi JC, Almeida FM, Nunes EC, Sadi MV (2008). "Residual urinary volume and urinary tract infection--when are they linked?". The Journal of Urology. 180 (1): 182–5.

Üçer O (2011). "Giant prostatic hyperplasia: Case report and literature review". Dicle Medical Journal / Dicle Tıp Dergisi. 38 (4): 489–491.

Ukwe C, Nwafor S (2005) Biology Nigerian Journal of Pharmaceutical research DOI:10.4314/NJPR.V3I1.35390. Corpus ID: 84326726

Varshney R, Kale RK (1990). Effects of calmodulin antagonists on radiation-induced lipid peroxidation in microsomes. International Journal of Radiation Biology. 58(5):733-43.

Verhamme KM, Dieleman JP, Bleumink GS, van der Lei J, Sturkenboom MC, Artibani W (2002). "Incidence and prevalence of lower urinary tract symptoms suggestive of benign prostatic hyperplasia in primary care--the Triumph project". European Urology. 42 (4): 323–8.

Vos T, Flaxman AD, Naghavi M, Lozano R, Michaud C, Ezzati M. (2012). "Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010". Lancet. 380 (9859): 2163–96.

Waigh RD (1998). The chemistry behind altracurium. Chemistry in Britain. 24: 120-129.

Wang J, Michelitsch T, Wunderlin A, Mahadeva R (2009). "Aging as a consequence of Misrepair –a novel theory of aging". 0904 (575).

Wang Y, Bao Y, Liu J, Duan L, Cui Y (2018). "Tadalafil 5 mg Once Daily Improves Lower Urinary Tract Symptoms and Erectile Dysfunction: A Systematic Review and Meta-analysis". Lower Urinary Tract Symptoms. 10 (1): 84–92.

Wang-Michelitsch J, Michelitsch T (2015). "Aging as a process of accumulation of Misrepairs". 1503 (7163).

Wasserman NF (2006). "Benign prostatic hyperplasia: a review and ultrasound classification". Radiologic Clinics of North America. 44 (5): 689–710, viii.

Wigg MD, Al-Jabri AA, Costa SS, Race E, Bodo B, Oxford JS (1996). In vitro virucidal and virustatic anti HIV-1 effects of extracts from *Persea americana* Mill.(avocado) leaves. Antiviral Chemistry and Chemotherapy. 7:179–83.

Wilt TJ, Mac Donald R, Rutks I (2003). "Tamsulosin for benign prostatic hyperplasia". The Cochrane Database of Systematic Reviews (1): CD002081.

Wilt TJ, MacDonald R, Ishani A. (1999). Beta-sitosterol for the treatment of benign prostatic hyperplasia: a systematic review. BJU International. 83(9):976-83.

Withering W. (1785). Therapeutic and toxic effects of digitalis. The Journal of Emergency Medicine Volume 4, Issue 3, 1986, Pages 243-248

Wu CP, Gu FL (1991). "The prostate in eunuchs". Progress in Clinical and Biological Research. 370: 249–55. PMID 1924456

Zhang SX, Yu B, Guo SL, Wang YW, Yin CK (2003). "Comparison of incidence of BPH and related factors between urban and rural inhabitants in district of Wannan". Zhonghua Nan Ke Xue = National Journal of Andrology. 9 (1): 45–7.

Zwart R, Verhaagh S, Buitelaar M, Popp-Snijders C, Barlow DP. (2001) Impaired activity of the extraneuronal monoamine transporter system known as uptake-2 in Orct3/Slc22a3-deficient mice. Mol Cell Biol 21:4188–4196.

APPENDIX I

Appendix 1: Calculations

Percentage yield:

$$percentate\ yield = \frac{waight\ of\ extracts}{waight\ of\ powder}\ x\ \frac{100}{1}$$

The volume of extract, standard drug or water to be administered to the animal is calculated thus:

$$volume\ = \frac{waight\ of\ animal\ \ (kg)\ x\ dose\ (mg/kg)}{concentration\ of\ drug\ (mg/kg)}$$

APPENDIX II

Groups	Dose	Superoxide Dismutase	Catalase	Glutathione Reductase	Glutathione Peroxidase	Malaldehyde
Negative Control	DW	2.27±0.23	41.80±4.80	2.43±0.27	55.43±3.84	63.30±6.68
Positive Control		5.17±0.15**	56.63±2.31*	3.67±0.24*	74.20±3.20	35.07±2.89**
Normal Control	Nil	1.50±0.17	28.87±1.63	1.80±0.26	45.47±3.36	75.03±4.75
Group 1	100mg/kg	7.40±0.84****	77.93±3.56****	4.60±0.32***	92.37±6.06**	24.33±1.82****
Group 2	200mg/kg	6.30±0.64***	71.27±1.68***	4.50±0.31***	93.60±8.52**	21.53±4.10****
Group 3	400mg/kg	6.33±0.45***	76.93±4.72****	4.90±0.06***	93.07±8.05**	15.53±0.38****

Table of Analysis

Results are represented as mean± standard error of mean (SEM) of each groups.

- * = $P \leq 0.05$
- ** = $p \leq 0.01$
- *** = $p \leq 0.001$
- **** = $p \leq 0.0001$