

EXPLOITING PLANT PARTS OF *Artocarpus heterophyllus* (JACK FRUIT TREE) FOR TREATMENT OF BENIGN PROSTATIC HYPERPLASIA IN MALE WINSTAR RATS

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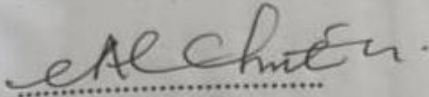
**A DISSERTATION SUBMITTED TO THE DEPARTMENT OF
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**IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE
AWARD OF DOCTOR OF PHILOSOPHY (Ph.D) DEGREE IN MEDICAL
BIOCHEMISTRY**

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CERTIFICATION

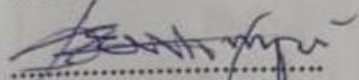
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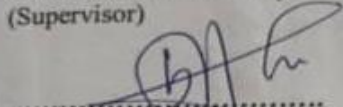
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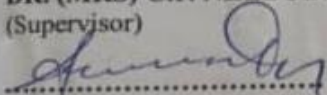
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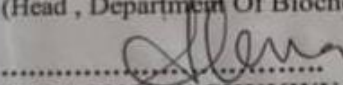
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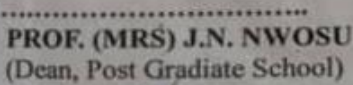
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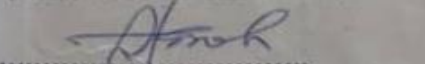
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DEDICATION

This work is dedicated to God Almighty and to the memory of my late Dad, Sir Benedict Nzekwe.

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ABSTRACT

Benign prostatic hyperplasia (BPH) is a common urological disorder affecting millions of men worldwide. This study employed a multi-analytical approach to evaluate the phytochemical, biochemical, nutritional, hematological, and histological properties of *Artocarpus heterophyllus* (jackfruit) stem bark, pulp, and leaf extracts, as well as their therapeutic effects in a testosterone propionate (TP)-induced prostate model in animals. Fifty five male winstar rats were divided into 11 groups of 5 rats each: Group 1 (normal control; feed and water only), Group 2 (negative control; BPH-induced with 5.0 mg/kg testosterone propionate), Group 3 (standard control; BPH-induced and treated with 5 mg/kg finasteride), and Groups 4–11 treated with various doses (250 or 500 mg/kg b.w.) of leaf, stem bark, pulp, or combined extracts for 21 days. Analysis of serum prostate-specific antigen (PSA) levels showed that the negative control group (1.34 ± 0.05 to 1.37 ± 0.08 ng/mL) exhibited no significant change, while TP-treated groups receiving extracts demonstrated significant reductions. Group 3 showed a PSA decrease from 2.98 ± 0.03 to 1.50 ± 0.11 ng/mL (~49.7%), Group 11 exhibited a similar reduction from 2.94 ± 0.08 to 1.48 ± 0.04 ng/mL (~49.7%), and Group 7 showed a decrease from 2.91 ± 0.05 to 1.53 ± 0.07 ng/mL (~47.4%), while moderate reductions occurred in Group 4 (2.98 ± 0.08 to 2.19 ± 0.16 ng/mL) and Group 8 (3.01 ± 0.04 to 2.29 ± 0.07 ng/mL). These results indicate dose-dependent and extract-specific effects likely mediated by flavonoids, tannins, and artocarpin through attenuation of oxidative stress, suppression of proinflammatory cytokines (e.g. IL-6). Acute toxicity testing confirmed safety up to 5000 mg/kg with no adverse behavioral or physiological effects. GC–MS identified 64, 94, and 48 compounds in stem bark, pulp, and leaf extracts respectively, including n-hexadecanoic acid, oleic acid, cis-vaccenic acid, tetradecanoic acid, and squalene, associated with antioxidant, anti-inflammatory, and anticancer activities. FTIR revealed hydroxyl, alkane, sulfur, nitro, nitrogenous, and aromatic functional groups across the extracts, indicating chemical diversity. Nutritional profiling showed proline (42.16 ppm) dominance in stem bark; threonine (16.39 ppm), isoleucine (10.06 ppm), and arginine (9.16 ppm) abundance in leaves; and high tyrosine (29.70 ppm), tryptophan (3.61 ppm), methionine (2.54 ppm), cystine (1.62 ppm), and glutamate (4.85 ppm) in pulp. Vitamins C, E, and A were highest in the leaf extract (22.14, 9.32, and 6.48 mg/100 g, respectively), alongside thiamine (0.45–0.61 mg/100 g), riboflavin (0.38–0.55 mg/100 g), and niacin (1.26–1.85 mg/100 g). Mineral analysis showed high potassium (215.64 mg/100 g), calcium (128.54 mg/100 g), and magnesium (87.61 mg/100 g), with trace zinc (2.48–3.11 mg/100 g) and iron (6.84–7.96 mg/100 g). Histology revealed normal hepatic structure in controls, severe degeneration in untreated toxicant groups, and hepatoprotection in extract-treated rats, especially pulp and stem bark extracts, which restored hepatocyte architecture. In the BPH model, untreated rats had elevated absolute (0.85 ± 0.21 g) and relative (0.43 ± 0.10 g) prostate weights compared to controls (0.41 ± 0.05 g and 0.24 ± 0.05 g), while stem and combination extracts (500 mg/kg) significantly reduced prostate weights to near-control levels, comparable to finasteride. These reductions were accompanied by lowered serum testosterone, PSA, TNF- α , and IL-6 levels, supporting antiandrogenic and anti-inflammatory mechanisms. Hematological results showed stable WBC counts ($99.90 \pm 0.00 \times 10^9/L$) with slight decreases in pulp (500 mg) and combination extract groups, elevated lymphocytes in the negative control ($54.30 \pm 3.52\%$) versus control ($27.67 \pm 0.81\%$), and increased hemoglobin (up to 18.30 ± 2.39 g/dL) and RBC counts (up to $14.80 \pm 3.20 \times 10^{12}/dL$) in treated groups. MCV, MCH, and MCHC values remained within normal ranges, and platelet counts ($8806\text{--}9933 \times 10^9/L$) were stable. Overall, the findings demonstrate that *A. heterophyllus* extracts possess significant antioxidant, anti-inflammatory, and anti-BPH properties, with strong safety profiles and diverse biochemical and nutritional potentials that support their therapeutic use against androgen-mediated prostate diso

Keywords: exploiting, plant, *Artocarpus heterophyllus*, treatment, benign prostatic, hyperplasia, winstar rats.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

Benign Prostatic Hyperplasia (BPH) is one of the most common non-malignant urological disorders in men, affecting both young and older populations worldwide. It is characterized by progressive enlargement of the prostate gland due to hyperplasia of stromal and epithelial cells, which compresses the urethra and obstructs urinary outflow. This mechanical obstruction leads to lower urinary tract symptoms (LUTS) including urinary frequency, urgency, weak stream, nocturia (night-time urination), and incomplete bladder emptying. These symptoms often impair quality of life, interfere with sleep, and increase the risk of secondary complications such as urinary tract infections and bladder dysfunction (McVary et al., 2021).

Epidemiological data show that BPH prevalence increases with age. According to the American Urological Association, about 50% of men in their 50s and nearly 90% of men in their 80s present with histological or clinical evidence of BPH. The Global Burden of Disease survey reported that BPH contributed to approximately 5.5 million disability-adjusted life years (DALYs) globally in 2012, underscoring its health and socioeconomic burden. In the United States, BPH accounts for more than two million physician visits annually and nearly 379,000 prostatectomies (Rencz et al., 2015). In sub-Saharan Africa, prevalence rates remain high; in Nigeria, about 25% of adult men were reported to have BPH (Ezeanyika, Ejike, Obidoa & Elom, 2006). Recent African surveys suggest an increasing trend, partly due to higher life expectancy and lifestyle transitions (Awe., Odugwu, Adebayo & Olapade, 2023).

The pathogenesis of BPH is multifactorial. Age-related hormonal changes play a central role, particularly the accumulation of dihydrotestosterone (DHT) in prostatic tissue. DHT is formed from testosterone via 5 α -reductase and binds to androgen receptors, driving transcriptional activity that promotes cellular proliferation and inhibits apoptosis. This androgen-mediated growth is exacerbated by altered estrogen-to-androgen ratios in older men, which sensitize prostatic cells to DHT (Xu, et al., 2024). Chronic inflammation has also been implicated, with immune infiltrates—macrophages, T cells, and cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- α)—triggering tissue remodeling, fibrosis, and stromal proliferation (McCance & Huether, 2018). Growth factors including fibroblast growth factor (FGF) and transforming growth factor-beta (TGF- β) further enhance extracellular matrix deposition and angiogenesis, fueling progressive glandular enlargement (Yin., Hu., Wang., Xu & Liu, 2022).

Emerging evidence highlights oxidative stress and mitochondrial dysfunction as critical drivers of BPH pathophysiology. These processes accelerate senescence in prostatic stromal and epithelial cells, disrupt genomic stability, and impair tissue homeostasis, thereby aggravating hyperplasia (Wang, Chen, Zhang & Xu, 2021). Moreover, metabolic disorders such as obesity, hypertension, insulin resistance, and type 2 diabetes have been strongly associated with BPH progression and LUTS severity, suggesting a link between systemic metabolic health and prostate growth (Russo et al., 2022).

Though BPH is not a malignant condition, it significantly impacts morbidity. If untreated, it may lead to complications such as acute urinary retention, recurrent urinary tract infections, bladder stones, hydronephrosis, and renal impairment (McVary et al., 2021). Standard treatments include pharmacological agents— α 1-adrenergic blockers and 5 α -reductase inhibitors—as well as surgical interventions such as transurethral resection of the prostate (TURP). However, these therapies are often associated with adverse effects such as dizziness, hypotension, decreased libido, erectile

dysfunction, infertility, and antibiotic resistance (Roehrborn, 2022). Consequently, there is a growing demand for alternative therapies that are both effective and safer.

Phytotherapy has attracted global interest as a promising alternative in the management of BPH. In Germany and Austria, phytotherapeutic agents account for about 90% of prescriptions for BPH management (Buck, 1996), reflecting their wide clinical acceptance. Plant-derived bioactive compounds possess anti-inflammatory, antioxidant, anti-proliferative, and anti-androgenic properties that target multiple mechanisms underlying prostate hyperplasia. Recent studies have highlighted *Serenoa repens*, *Pygeum africanum*, and *Urtica dioica* as effective plant-based therapies with favorable safety profiles (Russo et al., 2022; Saini, Arora, Kumar & Sharm,a 2023).

In tropical regions, plants such as *A. heterophyllus* (jackfruit) have shown considerable medicinal potential. Various parts of *A. heterophyllus* contain bioactive phytochemicals with documented anti-inflammatory, antioxidant, antibacterial, anticancer, and anti-proliferative properties (Hasmila, Natsir & Soekamto, 2019). Preliminary phytochemical screenings of its ethanol extracts revealed phenolic, flavonoid, and terpenoid compounds capable of modulating oxidative and inflammatory pathways. These attributes make *A. heterophyllus* a promising candidate for BPH therapy, with potential to provide safer, locally available, and cost-effective alternatives to conventional drugs. This study therefore seeks to explore the efficacy of *A. heterophyllus* ethanol extracts in ameliorating BPH, providing scientific evidence to support its ethnomedicinal applications.

1.2 Statement of Problem

Benign Prostatic Hyperplasia (BPH) is a critical public health issue due to its high incidence among men. Despite advancements in screening, diagnosis, and treatment, several key challenges remain in managing the disease effectively. *A. heterophyllus* is a known potent medicinal plant but, there is

limited research on the therapeutic effects and potential toxicity, of its parts in comparison to common drugs used in the treatment of the BPH.

1.3 Objective of the Study

The main objective of the study was to exploit the therapeutic and the toxicologic potentials of ethanol extracts of *A. heterophyllus* plants parts in the treatment of testosterone propionate-induced benign prostatic hyperplasia (BPH) in male winstar Rats.

The Specific objectives of this study were to:

1. Characterise and profile the phytochemical constituents present in selected plant parts of *A. heterophyllus*
2. Determine the phenolic compounds present in the plant parts
3. Determine the proximate content and fatty acid content of plant parts
4. Determine the Amino acid profile
5. Determine mineral and vitamin content of plant parts
6. Determine antioxidant and free radical scavenging potentials of the plant parts extract
7. Determine the acute toxicity of the plants parts
8. Induce experimental animals with benign prostatic hyperplasia
9. Evaluate the biomarkers of prostatic hyperplasia – Prostate Specific Antigen, Testosterone, Prostate weight.
10. Assess the anti-oxidative stress impact of plants parts extracts on winstar rats animals
11. Evaluate the toxicological effect of the plant parts extracts on on the biochemical parameters.
12. Evaluate the histology of prostate, liver and kidney

1.4 Justification of Study

There are different effective treatments for BPH, including medications, minimally invasive therapies, and surgery. However, there is the need to investigate the ameliorating potential of some medicinal herbs as a natural and cost-effective remedy on testosterone propionate-induced benign prostatic hyperplasia in male albino rats. Such common medicinal plants with various health benefits is the leaf, pulp, and stem bark of *A. heterophyllum*. Studies on *A. heterophyllum* show antibactericidal, antiprotozoal, insecticidal, antistress, Larvicide, anxiolytic, selective cytotoxicity of tumoral cells, wound healing, anti-ulcer, hypoglycaemic, hepatoprotective, and antioxidant properties among others. However, the ameliorating potentials of the combined plant parts of *A. heterophyllum* on testosterone propionate-induced benign prostatic hyperplasia in male albino rats is yet unknown and this necessitated this study

1.5 Scope of Study

The scope of this study included: plant collection and extraction of *A. heterophyllum* stem bark, leaves and fruits, Characterisation of ethanol extract of plant parts, proximate analysis, mineral, amino acid, vitamins, phenolics and antioxidants, preliminary acute toxicity studies, Animal handling, grouping, inductions and treatment with plant parts extracts, hematological, biochemical and histological analysis.

CHAPTER TWO

LITERATURE REVIEW

2.1 The Prostate

The prostate is part of the male reproductive system. It is the largest accessory gland in the male reproductive system. It secretes proteolytic enzymes into the semen, which act to break down clotting factors in the ejaculate. The proteolytic enzymes leave the prostate via the prostatic ducts. These open into the prostatic portion of the urethra, through 10-12 openings at each side of the seminal colliculus (or verumontanum); secreting the enzymes into the semen immediately before ejaculation. This allows the semen to remain in a fluid state, moving throughout the female reproductive tract for potential fertilization. The prostate is about the size of a walnut and weighs approximately one ounce. It sits below the bladder and in front of the rectum. It goes around a tube called the urethra. The urethra carries urine from the bladder and out through the penis (Prins, 2023). .

Traditionally, the prostate is divided into anatomical lobes (inferoposterior, inferolateral, superomedial, and anteromedial) by the urethra and the ejaculatory ducts as they pass through the organ. According to Ganzhe et al, the prostate is histological divided into three zones, namely:

Central zone – surrounds the ejaculatory ducts, comprising approximately 25% of normal prostate volume. The ducts of the glands from the central zone are obliquely emptying in the prostatic urethra, thus being rather immune to urine reflux.

Transitional zone – located centrally and surrounds the urethra, comprising approximately 5-10% of normal prostate volume. The glands of the transitional zone are those that typically undergo benign hyperplasia (BPH).

Peripheral zone – makes up the main body of the gland (approximately 65%) and is located posteriorly. The ducts of the glands from the peripheral zone are vertically emptying in the prostatic

urethra; that may explain the tendency of these glands to permit urine reflux. That also explains the high incidence of acute and chronic inflammation found in these compartments, a fact that may be linked to the high incidence of prostate carcinoma at the peripheral zone.

The fibromuscular stroma (or fourth zone for some) is situated anteriorly in the gland. It merges with the tissue of the urogenital diaphragm. This part of the gland is actually the result of interaction of the prostate gland budding around the urethra during prostate embryogenesis and the common horseshoe-like muscle precursor of the smooth and striated muscle that will eventually form the internal and external urethra sphincter.

Luminal cells are specialized cells that secrete a variety of products into the lumen, including prostate-specific antigen (PSA). Immunohistochemistry for PSA is strongly positive in luminal cells. The interstitium of the prostate is fibromuscular with abundant smooth muscle cells mixed with fibroblasts, blood vessels, and nerves. Notably, adipose tissue is absent in the prostate (Ganzhe , 2024)

The arterial supply to the prostate comes from the prostatic arteries, which are mainly derived from the internal iliac arteries. Some branches may also arise from the internal pudendal and middle rectal arteries. The prostate receives sympathetic, parasympathetic and sensory innervation from the inferior hypogastric plexus. The smooth muscle of the prostate gland is innervated by sympathetic fibres, which activate during ejaculation.

The prostate is flanked by the two neurovascular bundles that travel through the pelvic floor towards the penis, supplying it with nerve fibres and blood vessels for the corpora cavernosa. The integrity of these bundles is critical for normal erection.

During surgery for prostate cancer (radical prostatectomy), damage is often inevitable to one or both of these bundles, resulting in impairment of erectile function. Special nerve-sparing techniques may prevent extensive damage to these bundles, thus allowing for post-operative potency.

2.1.1 Prostate Specific Antigen (PSA)

Prostate-specific antigen (PSA) is a glycoprotein enzyme produced primarily by epithelial cells of the prostate gland. It plays a crucial role in male reproductive physiology. PSA is a serine protease belonging to the kallikrein family (specifically, kallikrein-related peptidase 3 or KLK3). It is secreted into the seminal fluid, where it functions to liquefy semen by breaking down high-molecular-weight proteins such as semenogelin (WebMD, 2025). This enzymatic activity facilitates sperm motility and fertility.

Under normal conditions, only small amounts of PSA enter the bloodstream. However, various prostate conditions can disrupt the prostate's cellular architecture, allowing more PSA to leak into the blood. These conditions include prostate cancer, benign prostatic hyperplasia (BPH), prostatitis, and prostate manipulation (WebMD, 2025). PSA has become a cornerstone in the detection and management of prostate-related diseases, particularly prostate cancer. Since its introduction in the late 1980s, the PSA test has significantly influenced prostate cancer screening and diagnosis (National Cancer Institute, 2024).

The PSA test measures the concentration of PSA in the blood, typically reported in nanograms per milliliter (ng/mL). While a threshold of 4.0 ng/mL has traditionally been used to prompt further investigation, this value is not definitive. Many men with PSA levels below this threshold may still have prostate cancer, and many with higher levels may not (National Cancer Institute, 2024). To improve diagnostic accuracy, clinicians may consider PSA velocity, PSA density, and the free vs. total PSA ratio. A meta-analysis found that PSA testing has high sensitivity (93%) but low specificity

(20%), indicating its usefulness in identifying potential cases but also its tendency to produce false positives (Ilic et al., 2018).

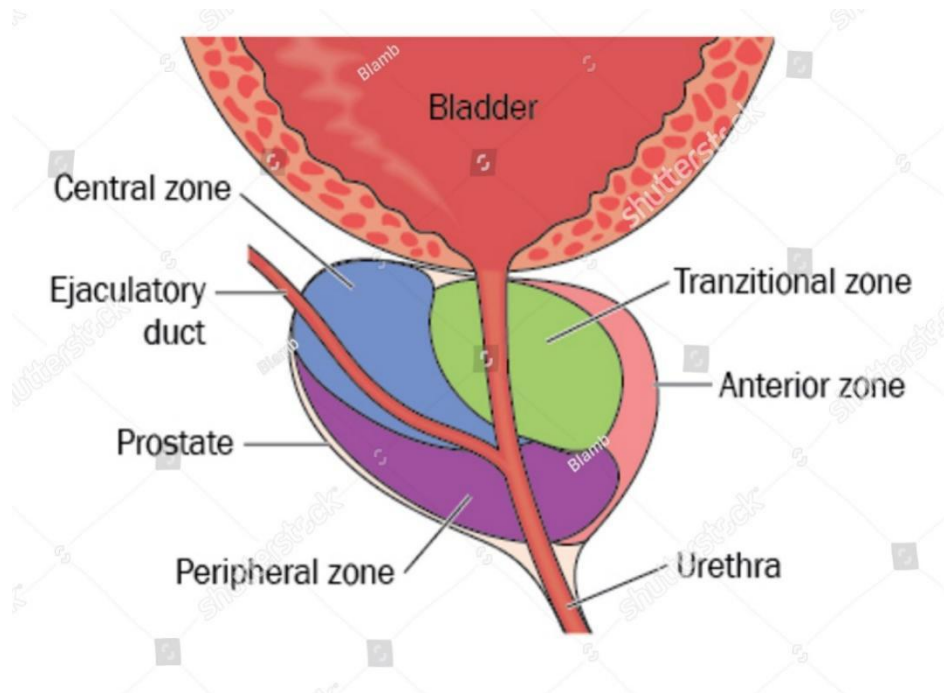


Figure 2.1: 4 zones of the prostate

(Source :medicine.net)

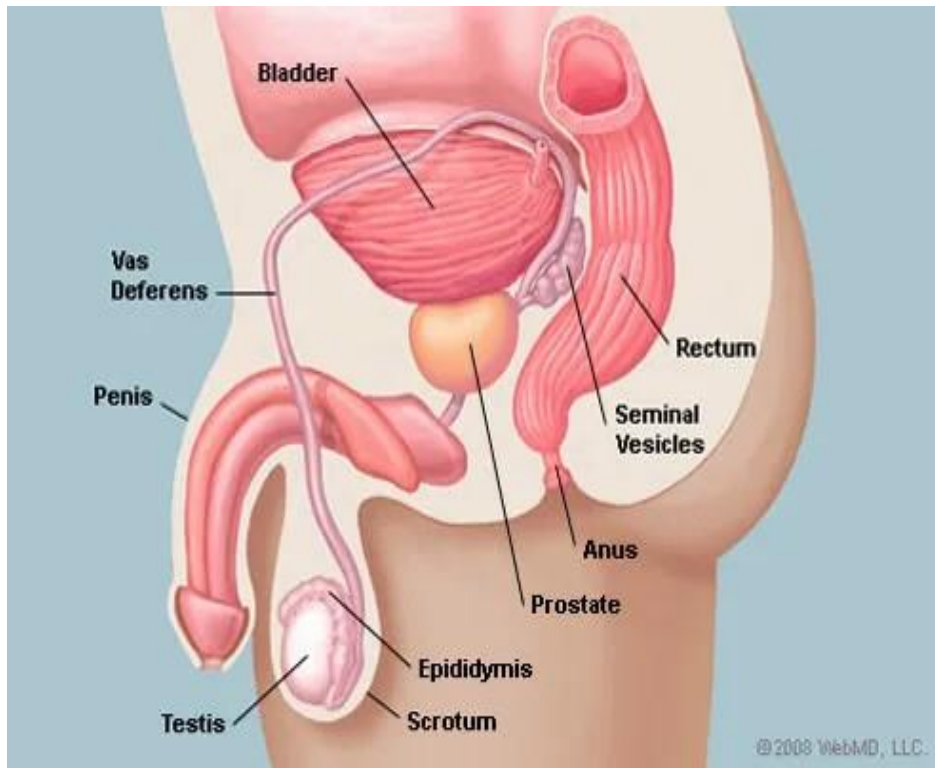


Figure 2.2 Side View of the Prostate (source:WebMD)

2.2 Benign Prostatic Hyperplasia (BPH)

Benign prostatic hyperplasia (BPH) is a condition in which the prostate gland grows larger than normal, but the growth is usually not cancerous. National Institute of Diabetes and Digestive and Kidney Diseases annual report, 2024 estimates, that BPH affects 5% to 6% of men ages 40 to 64 and 29% to 33% of those ages 65 and above. A prevalent neoplastic condition in older men, benign prostatic hyperplasia (BPH) has considerably lowered their quality of life and raised their risk of renal failure (Parsons & Kashefi, 2008). Prostate enlargement and symptoms related to the lower urinary tract are linked to the gradual proliferation of prostatic epithelial and stromal tissues brought about by increasing dihydrotestosterone stimulation. (Briganti et al., 2009). Prostate-specific antigen (PSA) concentrations and prostatic acid phosphatase (PAP) activity have been found to significantly increase

in BPH patients; these parameters are typically regarded as accurate indicators of the disease's progression (Ezugwu et al., 2018). There is increasing evidence that the primary cause of increased prostate cell development and hyperplasia in older men is an increase in concentration and sensitivity to dihydrotestosterone stimulation with aging (Iweala & Ogidigo, 2015). The most prevalent prostate issue in men over 50 is BPH, which hardly ever manifests symptoms in men under 40 (Langan, 2019). Symptoms include:

- trouble starting a urine stream or emptying the bladder
- a weak or interrupted urine stream, or dribbling at the end of urination
- nocturia
- urinary urgency
- urinary frequency
- pain during urination

2.2.1 Etiology of Benign Prostatic Hyperplasia (BPH)

Benign prostatic hyperplasia (BPH) is a noncancerous enlargement of the prostate gland due to androgenic stimulus exerted by dihydrotestosterone (DHT), a metabolite derived from testosterone by the action of the enzyme 5-alpha reductase. (Partin et al., 2021). The most important risk factors for developing BPH include age and the presence of functioning testicles (due to their hormonal influence); a family history of this condition and obesity (Ng & Baradhi, 2022).

Hormonal level changes with increasing age may also cause BPH. BPH arises due to the loss of homeostasis between prostatic cellular proliferation and apoptosis or cell death. This imbalance favors cellular proliferation without intervention. The result is increased numbers of prostatic periurethral epithelial and stromal cells, which can be seen histopathologically. (Roehrborn, 2008). The etiology

of BPH is influenced by a wide variety of risk factors, in addition to the direct hormonal effects of testosterone on prostate tissue. Men who are castrated before puberty or who have an androgen-related disorder do not develop BPH. (Ng , Leslie & Baradhi., 2024)

There is conflicting data on the role of non-steroidal anti-inflammatory medications (NSAIDs) in promoting BPH, with some studies indicating a positive association and others discounting any association. (Schenk et al., 2012). Allopurinol is somewhat protective for BPH, possibly secondary to reduced oxidative stress from hyperuricemia effects. (Kukko et al., 2018)

Testicular androgens are required to develop BPH as dihydrotestosterone (DHT) promotes tissue growth and cellular proliferation by interacting directly with prostatic epithelium and stroma. (Roehrborn 2008). Testosterone is converted to DHT by 5-alpha-reductase 2 in prostatic stromal cells and accounts for 90% of total intraprostatic androgens. (Chughtai et al., 2016). DHT directly influences prostatic stromal and adjacent cells, which affect cellular proliferation and apoptosis (Foster, 2009). Interestingly, there does not appear to be any relationship between testosterone or DHT levels and the development of symptomatic BPH. (Gann et al., 1995).

2.2.2 Risk Factors

Non-modifiable and modifiable risk factors also contribute to the development of BPH. These have been shown to include: diabetes, diet, genetic factors, localized inflammation, obesity, and metabolic syndrome. (Chughtai et al., 2016).

Diabetes and the use of antidiabetic medications, particularly insulin, appear to increase the risk of BPH, Lower Urinary Tract Syndrome (LUTS), and prostatic surgery. (Nygård et al., 2023; Rohrmann ,Smit, Giovannucci. & Platz , 2005).

Dietary factors also appear to influence the development of BPH. Beta-carotene, carotenoids, and vitamin A seem somewhat protective, while excessive alcohol ingestion, heavy caffeine intake, and high-dose supplemental vitamin C tend to increase BPH risk and symptoms. (Maserejian et al., 2013; Maserejian *et al.*, 2010). No prepared dietary supplement has been proven to help BPH in properly performed, randomized, controlled studies. (Ng et al., 2024).

Genetic Predisposition to BPH has been demonstrated in cohort studies. First-degree relatives in one study demonstrated a 4-fold increase in the risk of BPH compared to the control. (Sanda et al., 1994). These findings have demonstrated consistency in twin studies looking at the disease severity of BPH, with higher rates of LUTS seen in monozygotic twins. (Rohrmann et al., 2006).

Localized Inflammation is often associated with BPH, at least histologically. (Lloyd et al., 2019). While the exact etiology is unclear, possible causes include increased detrusor voiding pressure, obesity, low-grade or chronic prostatitis, compression of the prostatic ducts, and autoimmune disorders. This would suggest that Non-Steroidal Anti-inflammatory Drugs (NSAID) could be used to treat symptomatic BPH. Three randomized studies have confirmed that NSAIDs can improve BPH symptoms, but the difference was relatively modest at <3 points (International Prostate Symptom Scores [IPSS]) and <1 mL/s improvement in urine peak flow rate. (Kahokehr et al., 2013).

Obesity is associated with an increased risk of BPH in observational studies. (Kristal et al., 2007). The exact cause is unclear but is likely multifactorial, as obesity makes up one aspect of metabolic syndrome. Proposed mechanisms include increased levels of systemic inflammation and higher levels of estrogens (De Nunzio et al., 2012) the greater the amount of adiposity, the greater the prostate volume. Body weight, body mass index (BMI) and waist circumference have all been positively

associated with prostate volume in multiple different study populations. (Parsons,Sarma,McVary & Wei 2013).

Metabolic syndrome refers to conditions that include hypertension, glucose intolerance/insulin resistance, and dyslipidemia. Meta-analysis has demonstrated those with metabolic syndrome and obesity have significantly higher prostate volumes. (Gacci et al., 2015). Further studies looking at men with elevated glycosylated hemoglobin levels (Hb1c) have demonstrated an increased risk of LUTS. (Rohrmann et al., 2005). Limitations of these studies are that there were no subsequent significant differences in prostate symptom scores, and the effect of diabetes on LUTS has been shown to be multifactorial. (Gacci et al., 2015). Further studies are therefore required to establish causation in these individuals.

2.2.4 Epidemiology

Differences in definitions make the interpretation of population-based studies regarding BPH difficult. For example, BPH can refer to histology, prostate enlargement, prostatic glandular hypertrophy, bladder outlet obstruction, or just a physician's diagnosis of BPH. Lower urinary tract symptoms (LUTS) refer to the large variety of urinary symptoms shared by disorders affecting the prostate or bladder, which may or may not be due to BPH.

Age is a significant predictor of the development of BPH and subsequent LUTS. Fifty percent of men older than 50 show evidence of BPH, and the association with the development of LUTS increases linearly with age. (Loeb et al., 2009). This is supported by studies that have demonstrated increases in prostate volume with age (2% to 2.5% increase in size per year). (Loeb et al., 2009).

In the US, studies have shown BPH prevalence to be as high as 70% in those between 60 and 69 years of age and more than 80% in those over 70 years. (Wei, Calhoun, & Jacobson, 2005). In a Boston

area community health survey, the prevalence of male LUTS alone significantly increased with age from 8% (30 to 39 yrs) to 35% (60 to 69 yrs). Other US population-based studies have shown 56% of men between 50 and 79 years reported BPH symptoms. (Parsons et al., 2008).

At a population level, the reported prevalence of BPH increased dramatically between 1998 and 2007 in the US, with the number of cases nearly doubling. (Stroup, Palazzi-Churas, Kopp & Parsons, 2012). These increases are attributed to an aging population, with those older than 80 years projected to be 19.5 million in 2030 (up from 9.3 million in 2003). (CDC, 2003). As the worldwide population grows older, the number of symptomatic BPH cases is expected to rise.

Only few studies on BPH have been conducted in Nigeria and the International Prostate Symptom Score (IPSS) questionnaire were used. (Ojewola et al., 2017; Ezeanyika, Ejike, Obidoa & Elom, 2006). In different retrospective studies, as many as 25% and 12% of adult male Nigerians were reported to have BPH (Ezeanyika et al., 2006). Ojewola et al reported 57.4% in Southwest Nigeria in a prospective study, additionally, 71.0% was reported in another study in Southwest Nigeria (Ikuerowo et al., 2013). The wide range of prevalence rates in different studies may be attributed to variations in study design, population characteristics, and diagnostic criteria.

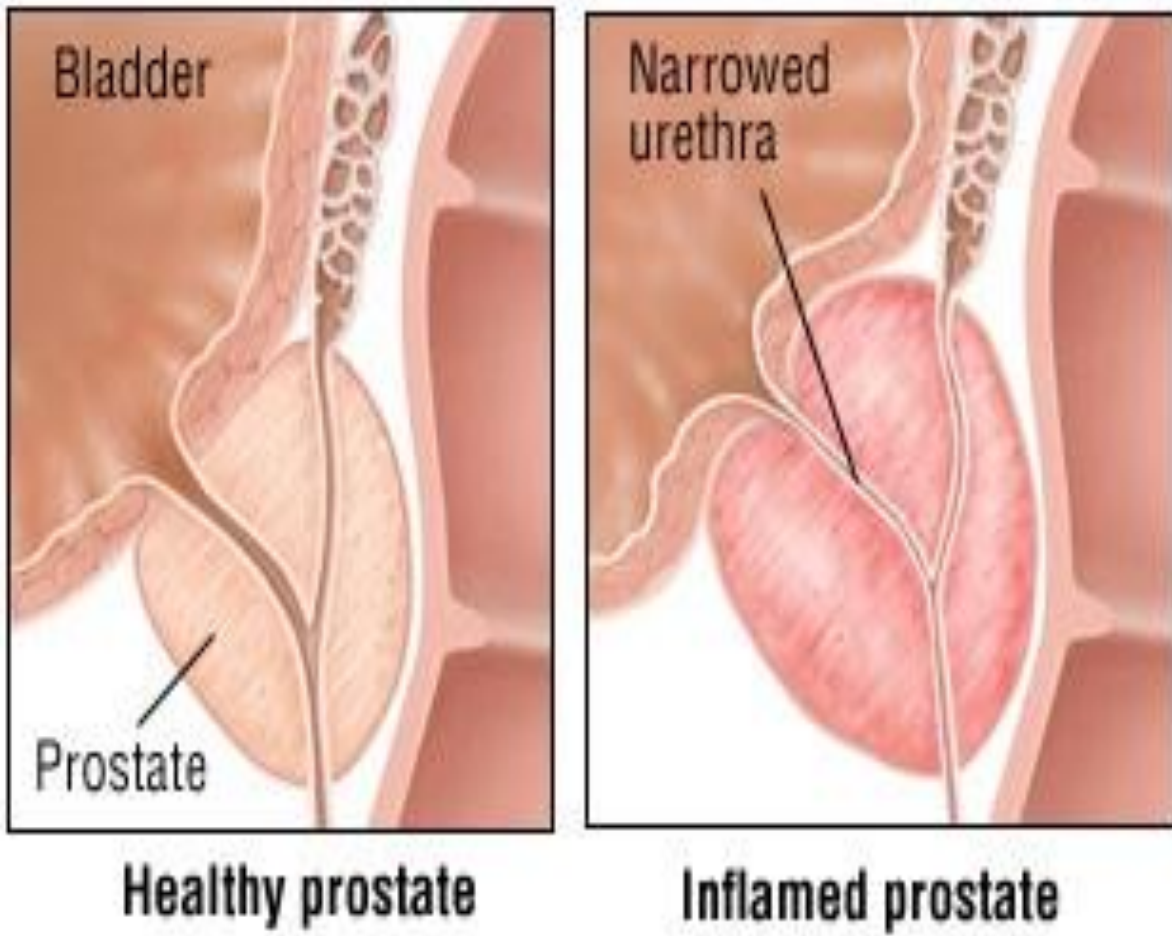


Figure 2.3 Healthy and Inflamed Prostate
(Source:medicine.net)

2.3 Medicinal Plants

Since the beginning of medicine, natural products—particularly those derived from plants—have been utilized to promote human health. For centuries, phytochemicals have served as remedies for a wide range of diseases and continue to provide essential leads for modern drug discovery. According to the World Health Organization, more than 80% of the global population in developing countries relies primarily on plant-based medicines for their basic healthcare needs (Canter , Thomas & Ernst, 2005). This reliance underscores the enduring role of medicinal plants as a primary healthcare resource, especially in regions where access to synthetic drugs is limited.

Scientific interest in the biological activities of plants has grown substantially due to the pharmacological significance of their bioactive constituents in both agriculture and medicine (Moghadamtousi et al., 2015). These natural compounds remain invaluable in inspiring the design of innovative and more effective pharmaceuticals for contemporary diseases (Johnson-Ajinwo & Nyodee, 2024). Historically, numerous breakthrough drugs originated from medicinal plants, including aspirin from *Salix alba* (willow bark), digoxin from *Digitalis purpurea* (foxglove), and artemisinin from *Artemisia annua* (sweet wormwood). Such examples demonstrate the critical role of ethnobotanical knowledge in guiding pharmaceutical research and development (Akbar, 2020). More recently, plant-derived compounds such as paclitaxel (from *Taxus brevifolia*) and vinca alkaloids (from *Catharanthus roseus*) have continued to revolutionize cancer therapy, further confirming the relevance of phytochemicals in modern medicine (Newman & Cragg, 2020).

Despite these successes, one of the major challenges in the medicinal plant sector remains the lack of standardized scientific validation. While traditional knowledge provides a useful foundation, rigorous preclinical screening, clinical trials, and toxicological studies are essential to establish safety and efficacy. Moreover, issues such as contamination, adulteration, misidentification, and inconsistent

dosage pose potential health risks (Kumar, 2022). The growing popularity of herbal supplements in both developed and developing countries raises additional concerns regarding product quality, regulation, and interactions with conventional drugs (Ekor, 2021).

Recent studies have emphasized the importance of standardization and the development of quality control frameworks for herbal medicines to enhance their credibility and integration into mainstream healthcare (World Health Organization, 2022; Sahoo, Manchikanti & Dey, 2023). Advances in metabolomics, molecular docking, and bioinformatics are also helping researchers unravel the mechanisms of action of phytochemicals, thereby bridging the gap between traditional medicine and evidence-based pharmacotherapy (Mensah et al., 2024). Furthermore, sustainable sourcing and conservation of medicinal plants have become urgent issues due to overharvesting, habitat loss, and climate change, highlighting the need for global policies that balance utilization with biodiversity preservation (Chen et al., 2023).

2.4 *Artocarpus heterophyllus* (Jack Fruit Tree)

The jackfruit (*Artocarpus heterophyllus*) belongs to the Moraceae family of trees, which also includes figs, mulberries, and breadfruits (Rahman, Chowdury, & Hassan 1999). *A. heterophyllus* Lam. Commonly known as jackfruit or “tree bread” in Latin America, a common name of *Artocarpus* genus like: *Artocarpus brasiliensis* Gomez., *Artocarpus heterophylla* Lam., *Artocarpus maxima* Blanco, *Artocarpus philippinensis* Lam., among others. Native to Southeast Asia and widely cultivated in Malaysia and the Western Ghats of India. (Praskash et al., 2009; Vazhacharickal et al., 2016; Nayak et al., 2017). The generic name comes from the Greek words ‘artos’ (bread) and ‘karpos’ (fruit); the fruits are eaten and are commonly called ‘African breadfruit’ or ‘Bread of the Tropics’. The specific name, ‘heterophyllus’, in Latin means, with leaves of different sizes and shapes and the word ‘heteros’ in Greek corresponds to the word ‘different’. The word ‘jackfruit’ comes from Portuguese jaca, which

in turn, is derived from the term ‘chakka’ in Malayalam language (Praskash et al., 2009). The ancient Indian Language Sanskrit refers this fruit as Atibruhatphala (Priyadarshani et al., 2007).

It most likely started in India's Western Ghats. Additionally, it is indigenous to Bangladesh and Malaysia, where it is the national fruit. Since its introduction and naturalization in many tropical regions, especially in South-Eastern Asia, the jackfruit has been grown for centuries (Morton, 1987). The jackfruit tree is extensively grown in tropical regions of the world and thrives in tropical lowlands. It is known in South East of Nigeria among the Igbos as *ukwa bekee/ukwa oyibo*. It is a perennial fruit tree crop, it grows massively on its branches and trunks , the tree can reach up to 8-25 meters in height and 2 meters in girth (Prakash et al., 2009).



Figure 2.4 A Jack Fruit Tree

Source:<https://www.istockphoto.com>

2.4.1 Morphology of *Artocarpus heterophyllus*

The tree *Artocarpus heterophyllus* (Jack fruit tree) is evergreen. It has deep roots in tap. Its dense, dome-shaped crown, which is rarely pyramidal, can reach a diameter of up to 6 meters in just five

years. The trunk is straight, rarely buttressed, with a girth of 50-100 cm. The stems are straight and make a 30° angle with the trunk, while the bark is rough, slightly scaly, and greyish-brown in color. Glabrous branches, twigs, and leaves release copious amounts of gummy latex when they are wounded. The simple, glossy, pinnately veined, alternating leaves are 4–25 cm in length by 2–12 cm in width. The underside of the leaves is pale green, while the upper side is dark green. The shape of the leaves can vary greatly depending on the tree's circumstances and maturity level.

The monoecious jackfruit tree bears solitary, axillary inflorescences on unique lateral, short, robust blooming shoots that emerge from the main trunk and older branches, or even from the base of the trunk in very ancient trees. The female flowers are solitary or paired, oblong or cylindrical, with rough, light to dark green skin, 5-15 cm long x 3-4.5 cm across, and encircled by a 5-8 cm long spathaceous bract. The male flowers are barrel-shaped or ellipsoid, pedicellated, and 3-8 cm long x 1-3 cm in diameter (Orwa et al., 2009; Morton, 1987). Large, pear-shaped, and cauliflorous, the fruit measures 20–100 cm in length and 15–50 cm in width. It could weigh anything from 4.5 kg to 25-50 kg. It grows along the trunk. Some varieties produce small fruits that weigh from 1.4 to 4.5 kg. The period between flowering and fruit ripening varies between 150 and 180 days (Jagtap & Bapat, 2010).

Each of the 500 indehiscent, fleshy, banana-flavored achenes that make up this fruit has a single, waxy, oval-oblong seed. When the fruit reaches maturity, its color changes from dark green to yellow to brownish. Hard pyramidal spines and a thick rubbery rind cover it. According to Orwa et al. (2009) and Morton (1987), when jackfruit reaches full maturity, its pulp smells of pineapple and banana, but the unopened fruit has a strong, repulsive scent similar to that of rotten onions.

Jackfruit is frequently picked early to prevent the big fruits from falling on people since fully mature fruit will fall off the tree. (Goswami., Hossain., Kader., & Islam, 2011). Like overripe fruit, the scent

of a mature jackfruit has been called "repungnant." The scent of younger, ripe fruit is sweeter. The flavor of jackfruit is sweet and reminiscent of bubblegum, pineapple, and bananas. The bulbs turn darker orange-yellow and the fruit's flavor becomes sweeter as it ages (Salim, Chin, and Kinghorn, 2008).

The fruit is tasty, aromatic, and succulent. It can be eaten fresh or preserved in a variety of ways. According to Sato and Fujiwara (1996), the nutrient-dense seeds can be prepared in meals, added to flour for baking, or boiled or roasted and consumed like chestnuts. It is renowned for its exceptional, long-lasting wood, which turns orange or reddish-brown with age. The fruit waste and leaves make excellent feed for goats, pigs, and cattle. The bark, roots, leaves, and fruit are among the many plant parts that are thought to have therapeutic qualities.

In Asia, jackfruit is a common fruit and a significant source of protein, vitamins, minerals, and energy. The pulp from immature fruit can be cooked as a vegetable or used in a variety of recipes, such as pickles and curries. Vegetarians and vegans enjoy it as a meat substitute because of its meat-like flavor and texture, which is evocative of chicken (Growables, 2020). In eastern and southern India, the fruit is referred to as the "poor man's fruit" since it is a staple vegetable and wholesome food. In various amounts, the seeds provide an inexpensive source of vitamins, minerals, carbs, lipids, fiber, vegetable oil, and high-quality protein. They are also very nutritious (Trease & Evans, 1989).

Numerous environmental benefits can be obtained from the tree. The thick jackfruit canopy is highly decorative and can serve as a visual screen in home gardens (Taylor, 1993).

One can utilize jackfruit trees to reduce soil erosion and floods. They serve as shade trees and windbreaks on coffee plantations or in stands where they give shade for cattle (Orwa, Mutua, Kindt, Jamnadass and Anthony 2009). Residential buildings in Thai cities have been shaded by jackfruit

trees, which have been shown to significantly reduce the structures' need for air conditioning (Akamphon & Akamphon., 2014).

2.5.3 Medicinal Uses

Traditional medicine has extensively utilized the fruits, leaves and bark of the jackfruit tree due to their different therapeutic activities. The plant has been shown to possess anticarcinogenic, antibacterial, antifungal, anti-inflammatory, wound-healing and hypoglycemic properties (Ranasinghe., Maduwanthi., & Marapana 2019)

All parts of jackfruit tree are used as traditional medicine. They are recommended for the treatment of inflammation, malarial fever, kidney stones, ulcers, infected wounds, diarrhea, fever, asthma, anemia, and dermatitis (Jagtap & Bapat 2010), as well as soothing . The seeds are used to heal sexual disorders due to its aphrodisiac properties (Fonseca, 2016). Scientific evidence of the healing properties of different parts of the jackfruit tree has already been presented. Particular emphasis has been given in the literature to the antioxidant activity from leaf, bark, and fruit extracts (Loizzo et al., 2010; Omar et al., 2011), jacalin and artocarpin antiviral activity (Tamma et al., 2003), anticancer activity of artocarpin (Septama & Daud, 2021), anti-inflammatory activity of flavonoids isolated from the bark (Wei et al., 2005), and antibacterial (Khan et al., 2003) and antifungal potential (Trindade et al., 2006) of several extracts and fractions.

This study will investigate the usefulness of combinations of the pulp, leaf and stem bark of extracts of *A. heterophyllus* in the management of the experimentally testosterone-induced BPH in male adult albino rats. The results will contribute to the search for locally available phytotherapeutic agent that can help in managing this debilitating disease especially among the less privileged.

2.5.4 Pharmacological activities of *Artocarpus heterophyllus*

Artocarpus heterophyllus Lam. (jackfruit) is a multi-purpose tropical plant whose various organs (leaves, fruits, seeds, latex, bark, and roots) are rich in phytochemicals such as phenolics, flavonoids, stilbenoids, prenylated flavones (e.g., artocarpin), sterols (β -sitosterol), and polysaccharides. These bioactive compounds underlie its broad spectrum of biological activities. Recent preclinical and phytochemical work has reinforced jackfruit's potential as a source of antioxidant, anti-inflammatory, antimicrobial, antidiabetic, and anticancer agents (Rodríguez-Espinoza et al., 2021; Natta et al., 2023; Morrison et al., 2021).

2.5.4.1 Antioxidant activity

Multiple studies have consistently reported strong antioxidant activity across different tissues of *Artocarpus heterophyllus*, including the fruit pulp, peel, leaves, and seeds. These effects are largely attributable to its high total phenolic and flavonoid content, with major bioactive constituents such as artocarpin, norartocarpetin, and cycloartocarpin contributing significantly to its free radical-scavenging potential (Rodríguez-Espinoza et al., 2021; Natta et al., 2023). Extracts from various plant parts have demonstrated dose-dependent radical scavenging activity in in vitro assays such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), as well as ferric reducing antioxidant power (FRAP) assays. In addition to direct free radical neutralization, these extracts have shown the capacity to protect cellular biomolecules from oxidative injury by inhibiting lipid peroxidation, enhancing glutathione levels, and upregulating endogenous antioxidant enzymes like superoxide dismutase (SOD) and catalase in experimental models (Morrison et al., 2021; Yuniarti et al., 2025).

Importantly, the antioxidant effects of *A. heterophyllus* extend beyond *in vitro* studies. *In vivo* investigations have demonstrated its ability to attenuate oxidative stress-induced tissue injury in liver,

kidney, and cardiovascular models, suggesting systemic benefits (Soni et al., 2024). For instance, jackfruit leaf extract reduced malondialdehyde (MDA) levels—a marker of lipid peroxidation—while improving antioxidant defense in diabetic rat models, indicating its potential role in mitigating oxidative stress–related complications of chronic diseases (Yuniarti et al., 2025).

2.5.4.2 Anti-inflammatory and immunomodulatory effects

Jackfruit extracts and isolated constituents, particularly artocarpin, have demonstrated pronounced anti-inflammatory activity across both *in vitro* and *in vivo* studies. These effects are mediated through multiple pathways, most notably the suppression of pro-inflammatory cytokines such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF- α), and interleukin-1 β (IL-1 β), which are central mediators of chronic inflammation (Morrison et al., 2021). In experimental colitis models, jackfruit leaf and seed extracts were reported to attenuate colonic inflammation by reducing inflammatory cell infiltration, downregulating cyclooxygenase-2 (COX-2) expression, and inhibiting nitric oxide (NO) production. Such modulation of inflammatory mediators provides a mechanistic basis for its potential use in managing gastrointestinal inflammatory disorders.

Beyond gastrointestinal models, *A. heterophyllus* has also shown protective effects in systemic inflammation. For example, extracts reduced edema formation and vascular permeability in carrageenan-induced paw inflammation, demonstrating classical anti-inflammatory potential comparable to standard drugs (Natta et al., 2023). Additionally, artocarpin has been shown to inhibit nuclear factor kappa B (NF- κ B) activation and mitogen-activated protein kinase (MAPK) signaling pathways, both of which regulate transcription of pro-inflammatory genes (Yuniarti et al., 2025).

Emerging evidence also supports chemopreventive activity of jackfruit-derived compounds against inflammation-driven tumorigenesis. Chronic inflammation is a well-established risk factor for cancer development, and by dampening inflammatory signaling, jackfruit flavonoids and prenylated

phenolics may reduce the progression of premalignant lesions. Furthermore, immunomodulatory properties have been reported, including modulation of leukocyte activity and enhancement of macrophage phagocytosis, suggesting that *A. heterophyllus* can balance immune responses—suppressing excessive inflammation while promoting adequate host defense (Soni et al., 2024; Yuniarti et al., 2025).

2.5.4.3 Antimicrobial and antibiofilm activity

Extracts of jackfruit seeds, leaves, latex, and bark have consistently demonstrated potent antibacterial and antibiofilm activities against both Gram-positive and Gram-negative pathogens. Methanolic and ethanolic extracts exhibited strong inhibitory effects on bacterial growth and biofilm formation, particularly against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* (Natta et al., 2023). These effects are often attributed to phytochemicals such as phenolics, flavonoids, and prenylated chalcones, which can disrupt bacterial cell membranes, inhibit enzyme activity, and interfere with quorum-sensing mechanisms (Soni et al., 2024).

Notably, jackfruit seed extracts have been shown to inhibit *P. aeruginosa* biofilm development by reducing exopolysaccharide production and suppressing quorum-sensing virulence factors, thereby enhancing susceptibility to conventional antibiotics (Kumar et al., 2022). In addition, the latex of *A. heterophyllus* displayed broad-spectrum antibacterial activity against multidrug-resistant clinical isolates, supporting its potential as an alternative therapeutic option in combating antimicrobial resistance (Pradhan et al., 2021). Furthermore, the bark extract exhibited synergistic effects when combined with standard antibiotics, indicating a possible role in reducing antibiotic resistance and enhancing treatment efficacy (Rahman et al., 2023).

2.5.4.4 Antidiabetic and metabolic effects

Unripe fruit and leaf extracts of *Artocarpus heterophyllus* have demonstrated significant antihyperglycemic activity, primarily through the inhibition of key carbohydrate-hydrolyzing enzymes such as α -amylase and α -glucosidase, thereby delaying intestinal glucose absorption and postprandial hyperglycemia (Rodríguez-Espinoza et al., 2021; Yuniarti et al., 2025). In vivo studies in streptozotocin-induced diabetic rats have shown reductions in fasting blood glucose, glycosylated hemoglobin (HbA1c), and improvements in oral glucose tolerance tests following administration of jackfruit seed and leaf extracts (Chandra et al., 2022).

Moreover, extracts have been linked to pancreatic β -cell preservation and regeneration, possibly mediated by their antioxidant-rich phytoconstituents, including flavonoids, phenolics, and artocarpin, which mitigate oxidative stress-induced apoptosis in islet cells (Yuniarti et al., 2025). *Drosophila melanogaster* models of diabetes further revealed improved survival rates, normalized triglyceride levels, and reduced glucose accumulation in hemolymph, supporting the systemic metabolic benefits of jackfruit bioactives (Rahman et al., 2023).

Additional reports highlight improved lipid metabolism, with reductions in total cholesterol, triglycerides, and low-density lipoprotein cholesterol (LDL-C), alongside elevations in high-density lipoprotein cholesterol (HDL-C), suggesting both glycemic and cardioprotective roles of jackfruit extracts (Mohan et al., 2024).

2.5.4.5 Anticancer and chemopreventive potential

Jackfruit leaf and seed extracts have demonstrated promising cytotoxic and chemopreventive activities against multiple cancer models. In vitro studies revealed that both methanolic and ethanolic extracts significantly inhibited the proliferation of colon (HT-29) and breast (MCF-7) cancer cell lines in a dose-dependent manner, primarily through the induction of apoptosis and cell cycle arrest

(Morrison et al., 2021). The flavonoid artocarpin, one of the most studied bioactive compounds of *A. heterophyllus*, has been shown to modulate tumor-related pathways, including the PI3K/Akt and MAPK signaling cascades, leading to downregulation of anti-apoptotic proteins (Bcl-2, survivin) and upregulation of pro-apoptotic markers such as caspase-3 and Bax (Yuniarti et al., 2025).

Additionally, jackfruit extracts exhibit significant effects in inflammation-associated carcinogenesis models, where chronic inflammation accelerates tumorigenesis. Treatment with leaf and bark extracts reduced tumor burden and suppressed inflammatory mediators such as TNF- α , IL-6, and cyclooxygenase-2 (COX-2), thereby disrupting the tumor microenvironment and preventing malignant transformation (Rahman et al., 2023). The antioxidant properties of jackfruit phytochemicals further contribute to its chemopreventive action by reducing oxidative DNA damage, enhancing repair pathways, and protecting cellular integrity (Rodríguez-Espinoza et al., 2021).

Beyond apoptosis and anti-inflammatory mechanisms, recent evidence also highlights the anti-metastatic potential of jackfruit-derived compounds. Extracts from seeds and bark inhibited epithelial-mesenchymal transition (EMT) markers such as vimentin and N-cadherin, while promoting E-cadherin expression, thereby reducing invasiveness and metastatic potential of cancer cells (Chandra et al., 2022).

2.5.4.6 Nutritional, prebiotic, and other activities

Beyond its pharmacological effects, *Artocarpus heterophyllus* (jackfruit) also provides considerable nutritional and technological benefits. Nutritionally, jackfruit is rich in dietary fibre, essential vitamins (such as vitamin C, vitamin A, and several B-complex vitamins), and minerals including potassium, magnesium, and calcium, which contribute to cardiovascular health, gut integrity, and overall metabolic function (Swami et al., 2021). Its high carbohydrate and protein content make it a valuable

source of energy. At the same time, its seeds are a rich source of resistant starch and micronutrients that can improve satiety and glycemic control (Reddy *et al.*, 2022).

Emerging evidence highlights the prebiotic potential of jackfruit polysaccharides, which support the growth of beneficial gut microbiota such as *Bifidobacterium* and *Lactobacillus*, thereby enhancing gut health and immunity (Natta *et al.*, 2023). This aligns with its increasing application as a functional food ingredient in bakery, confectionery, and nutraceutical formulations (Patel *et al.*, 2024). Moreover, jackfruit's antioxidant-rich extracts have shown potential in improving lipid metabolism and reducing oxidative stress when incorporated into dietary interventions.

Beyond nutrition, jackfruit also holds technological value. Its peel, seeds, and other by-products, which are often discarded, have been explored in the green synthesis of metallic nanoparticles (silver, zinc oxide, and gold nanoparticles). These eco-friendly nanoparticles exhibit antimicrobial, antioxidant, and catalytic activities, suggesting their use in biomedical, environmental, and food preservation applications (Rodríguez-Espinoza *et al.*, 2021; Gupta *et al.*, 2023). Such biotechnological applications not only add economic value to jackfruit waste but also contribute to sustainable practices in nanotechnology.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Equipment

Automated haematology analyzer (EMA D-6031 model, Bioevopeak, China), rotary evaporator (BuchiRotavapour-Switzerland), magnetic stirrer, refrigerator, flame photometer, spectrophotometer (Genesys 10-S, USA) , water bath (Grant, England), centrifuge(Clay Adams ,USA), Agilent Technologies gas chromatography equipment, (GC-7890A model coupled with an MS-5975C detector)

3.1.2 Reagents

The chemicals and reagents included ALT , AST and ALP test kits (Randox) , Alkaline phosphatase test kit (Randox), Bilurubin test kit (Randox), Total protein kit(Radox), Ethanol (BDH, England) , Distilled water, DPPH – 2,2-diphenyl-2-picryl hydrazyl hydrate, Phosphate buffer, H₂O₂, EDTA, nitroblue, tetrazolium, riboflavin, Sodium nitroprusside, Phosphate buffered saline, Griess reagent, Deoxyribose, Ferric chloride, Ascorbate, KH₂PO₄-KOH buffer, Thiobarbituric acid, TCA, TBA, Phenazine methosulphate, Nitroblue tetrazolium, NADH, Glacial acetic acid, Potassium phosphate buffer, DTNB, Standard GSH, Potassium ferrocyanide, trichloroacetic acid, ferric chloride, Folin-Ciocalteau reagent, Sodium carbonate, Standard catechol solution, Thiobarbituric acid, sodium hydroxide, glacial acetic acid, ammonium molybdate, disodium hydrogen phosphate solution, DTNB, chromogen reagent, formal saline, Sodium pyrophosphate buffer (0.025M, pH 8.3), Phenazine methosulphate (PMS) (186 μM), Nitroblue tetrazolium (NBT) (300 μM), NADH (780 μM), n-butanol, Potassium phosphate buffer (50mM, pH 6.4). All chemicals and reagents were of analytical grade.

3.1.3 Sample Collection and Authentication

The stem bark, leaves and pulp of *A. heterophyllus* were collected from the premises of Federal University of Technology, Owerri (FUTO). The leaves were identified by a plant taxonomist, Mr Iwueze Francis of The Department of Forestry And Wildlife Technology, School Of Agriculture And Agricultural Technology Federal University Of Technology Owerri with voucher number: FUTO/FWT/ERB/2025/143

3.2 Methods

3.2.1 Sample preparation

The evaluated samples were prepared separately according to standard operating protocol. Individual samples were washed, air-dried at room temperature for four (4) weeks and pulverised via blending, and stored in airtight inert container till use.

3.2.2 Extraction Of Plant Material

Extraction was done by the maceration method. 500g of each plant part powder was soaked separately in 750ml ethanol for 3 days, and was then filtered. The excess solvent was removed under vacuum using a rotary evaporator at 40 °C. Evaporation was continued to near dryness using a water bath at 40 °C. The crude was left to completely evaporate to dryness at room temperature in a beaker covered with clean, thinly perforated foil, and the crude extract was kept in an airtight inert container and refrigerated at 4°C till use.

3.2.3 Chemical characterization of plant samples using gas chromatography-mass spectroscopy (GC-MS)

To identify bioactive compounds, the extract underwent GC-MS analysis using Agilent Technologies gas chromatography equipment, as per Dike et al.'s methodology. The analysis was conducted using a GC-7890A model coupled with an MS-5975C detector, which featured an HP-5MS capillary

column (30 m length, 250 μm internal diameter, 0.25 μm film thickness). Electron ionisation at 70 eV was used for spectral detection. Helium gas, with a purity, was used as the carrier at a flow rate of 1 mL/min. The temperature of the oven began at 50 $^{\circ}\text{C}$, increased to 150 $^{\circ}\text{C}$ at 3 $^{\circ}\text{C}/\text{min}$, and was maintained for 10 minutes. Afterward, it was raised to 300 $^{\circ}\text{C}$ at a rate of 10 $^{\circ}\text{C}/\text{min}$. A 1 μL aliquot of a 1% extract solution, dissolved in a suitable solvent, was introduced via splitless injection. The percentage of each compound in the extract was based on peak areas from the chromatogram (Dike et al., 2023).

3.2.4 Identification of chemical constituents

Bioactive compounds within the extract were pinpointed through gas chromatography, a technique that scrutinised their retention times using an HP-5MS column. Subsequently, the mass spectra derived from this process were juxtaposed against authoritative spectral databases, Replib and Mainlib, integral to the GC-MS system, enabling definitive compound identification (Dike et al., 2023).

3.2.4 FTIR Analysis

Fourier Transform Infrared analysis was performed utilizing a Buck Scientific M530 instrument, which includes a deuterated triglycine sulfate detector and a potassium bromide beam splitter. The Gram A1 software was used for acquiring and processing spectral data. Samples were prepared by mixing 1.0 g of the extract with 0.5 mL of nujol, followed by application onto a salt pellet. Infrared spectra were then recorded between 4,000 and 600 cm^{-1} . Spectral data were obtained through the co-addition of 32 scans at a 4 cm^{-1} resolution, with transmittance values representing the resultant spectra (Dike et al., 2023).

3.2.5. Proximate Analysis

3.2.5.1 Moisture content determination

The moisture content was easily determined by the drying method in the sample. 2 g of sample was taken (initial weight). The sample was placed in an oven at 105°C temperatures for 12 hours-24 hours and its weight was recorded after operation (final weight). The following formula was used to calculate the percentage of moisture:

$$\% \text{ moisture content} = \frac{W1 - W2}{\text{Weight of sample}} \times 100 \quad 3.1$$

Where w1 = weight of petri dish and sample before drying

W2 = weight of petri dish and sample after drying (AOAC 1995).

3.2.5.2 Ash content determination

This was done using the method of AOAC, (1995). Briefly, empty platinum crucible was washed, dried and the weight was noted. Approximately 2g of sample was weighed into the platinum crucible and placed in a muffle furnace at 550⁰c for 3 hours. The sample was cooled in a dessicator after burning and weighed. The percentage ash content was calculated thus;

$$\% \text{ Ash content} = \frac{W3 - W1}{W2 - W1} \times 100 \quad 3.2$$

Where

W1 = weight of empty platinum crucible

W2 = weight of platinum crucible and sample before burning

W3 = weight of platinum and ash.

3.2.5.3 Crude fibre determination

This was done using the method of AOAC, (1995). Briefly, 2g of material was defat with petroleum ether, and boiled under reflux for 30 minutes with 200ml of a solution containing 1.25g of H₂SO₄ per 100ml of solution. The solution was filtered through linen, and washed with boiling water until the washings are no longer acid. The residue was then transferred to a beaker and boiled for 30 minutes with 200ml of a solution containing 1.25g of carbonate free NaOH per 100ml. the final residue was filtered through a thin but close pad of washed and ignited asbestos in a Gooch crucible. It was dried in an oven, weighed, incinerated, cooled, and weighed. The percent loss in weight after incineration is calculated as the crude fibre.

$$\% \text{crude fibre} = \frac{\text{weight of Fibre}}{\text{weight of sample}} \times 100$$

3.3

3.2.5.4 Determination Of Crude Fat

This method of Onuoha (2018) was carried out by continuously extracting with petroleum ether for at least 1 hour. 250ml clean boiling flask was dried in oven at 105 – 110 °c for about 30 minutes. It was allowed to cool in a dessicator. The boiling flask was filled with about 300 ml of petroleum ether (boiling point 40 - 60⁰c). The extraction thimble was plugged lightly with cotton wool, and the soxhlet apparatus was assembled, and allowed to reflux for about 6 hours. The thimble was carefully removed, and the petroleum ether in the top container of the set – up and drain into a container for re – use. The flask was removed and dried at 105⁰c - 110⁰c for 1hour when it was almost free of petroleum ether. It was transferred from the oven into a dessicator, allowed to cool, and was then weighed. The percentage fat was calculated thus;

$$\% \text{ fat} = \frac{(\text{wt of flask + oil}) - \text{wt of flask}}{\text{wt of sample}} \times 100$$

Wt of sample 1 3.4

3.2.5.5 Determination Of Crude Proteins

The crude protein was determined by micro Kjeldahl technique, as described by Moses et al., (2012). In a heating tube, two grams of materials were dissolved in 10 mL of concentrated H₂SO₄. It was heated inside a fume cupboard with 1 Selenium tablet as a catalyst. The mixture was then shifted to a 100 mL flask and the volume was increased with distilled water. 10 mL of the mixture was mixed with an equivalent volume of 45% NaOH solution before being relocated to the Kjeldahl apparatus. The extract was combined with 4% H₃BO₃ and 3 droplets of methyl red as an indicator. A sample of 50 mL of distillate was obtained and titrated. The test was repeated three times, and the mean value was computed. The nitrogen content was calculated and multiplied by 6.25 to get crude protein content, thus;

$$CP (\%) = \frac{(T-B) \times N \times 14 \times 100 \times 6.25}{W_s \times 1000} \quad 3.5$$

3.2.5.6 Carbohydrate Content Determination

The percentages of carbohydrates were found out by the Nitrogen-free approach. The carbohydrate amount was calculated by subtracting the total sum of other proximate characteristics such as crude fat, crude protein, crude fiber, ash, and moisture from 100. The formula is as follows:

$$Carbohydrates (\%) = 100 - (\% \text{ ash} + \% \text{ Moisture} + \% \text{ Fats} + \% \text{ Fibres} + \% \text{ Protein}) \quad 3.6$$

(Ullah et al., 2023).

3.2.6 Fatty Acid Profile Analysis

This was done according to the method of Brockmann et al. (1974).

One thousand miligram (1000 mg) of sample was weighed into a 25 mL centrifuge tube, and 2 ml of water was added and mixed to dissolve. The mixture was let to sit for 15 minutes at room temperature.

5 mL of internal standard (C11:0 FAME + C13:0 TAG, each at 2 mg/mL in methyl tert butyl ether) was added. 5 mL of 5% (w/v) methanolic sodium methoxide solution was also added, and the tube was closed and vortex for 10 seconds. After 180 seconds from when sodium methoxide was added, 2 mL of hexane was added, and after 210 seconds, 10 mL of neutralization solution (10% disodium hydrogen citrate/15% sodium chloride in water) was added, and shaken gently using vortex mixer. The mixture was centrifuged at 1,750 rpm for 5 minutes. 200 μ L of supernatant was transferred into 10 mL flask and diluted to mark with hexane.

3.2.7 Amino Acid Determination

HPLC apparatus

The HPLC equipment consisted of a Spectra Physics (San Jose, CA) HPLC apparatus comprising an 8700 XR ternary pump, a 20- μ L Rheodyne (Cotati, CA) injection loop, an SP8792 column heater, a 8440 XR UV-vis detector, and a 4290 integrator linked via Labnet to a computer running WINner 8086 software (operating system, MS.DOS version 3.2). For separation, a 250- $\times$ 4.6-mm column packed with 5- μ m Spherisorb C₁₈ (Sugelabor, Madrid, Spain) was used.

3.2.7.1 Preparation of Samples and Standards

Prior to derivatization, sample proteins were hydrolyzed as follows. A 0.1-g lyophilized sample was weighed into a 16- $\times$ 125-mm screw-cap Pyrex (Barcelona, Spain) tube, 15 mL of 6N hydrochloric acid was added, and the tube was thoroughly flushed with N₂, quickly capped, and placed in an oven at 110°C for 24 h. After hydrolysis, the tube contents were vacuum filtered to remove solids, the filtrate was made up to 25 mL with water, and an aliquot of this solution was further filtered through a 0.50- μ m pore-size membrane. A standard solution containing 1.25 μ mol/mL of each amino acid in 0.1N hydrochloric acid was created.

3.2.7.2 Derivatization Procedure

The procedure used was a modification of the method of Elkin et al (1989). A standard solution (5, 10, 15, or 20 μL) or 50 μL of sample solution was pipetted into a 10- $\times$ 5-mm tube and dried in vacuo at 65°C. To the residue, 30 μL of methanol-water-Phenylisothiocyanate (2:2:1 [v/v]) was added and then removed in vacuo at 65°C. Next, 30 μL of the derivatizing reagent methanol-water-Phenylisothiocyanate (7:1:1:1 [v/v]) was added, and the tube was agitated and left to stand at room temperature for 20 min. Finally, the solvents were removed under a nitrogen stream, and the tube was sealed and stored at 4°C, pending analysis. Prior to injection, 150 μL of diluent consisting of 5mM sodium phosphate with 5% acetonitrile was added to each tube.

3.2.7.3 Chromatographic Procedure

Chromatography was carried out at a constant temperature of 30°C using a gradient elution as follows. Eluant A was an aqueous buffer prepared by adding 0.5 mL/L Triethylamine to 0.14M sodium acetate and titrating it to pH 6.20 with glacial acetic acid; eluant B was acetonitrile-water (60:40 [v/v]).

Table 3.1: Gradient Program Employed for Separation of Amino Acids

Time Flow rate			
(min)	(mL/min)	% Eluent A	% Eluent B
0	1.0	90	10
12.0	1.0	70	30
20.0	1.0	52	48

22.0	1.0	0	100
24.0	1.0	0	100
30.0	1.5	0	100
37.0	1.0	90	10

3.2.8 Vitamin Analysis

3.2.8.1 Vitamin A estimation

Vitamin A was estimated by the method of Bayfield and Cole (1980). All procedures were carried out in the dark to avoid the interference of light. 1g of sample was mixed with 1.0ml of saponification mixture and refluxed for 20 minutes at 60 °C in the dark. The tubes were cooled, and 20ml of water was added and mixed well. Vitamin A was extracted twice with 10ml of (40°-60°C) petroleum ether. The two samples were pooled and washed thoroughly with water. Anhydrous sodium sulphate was added to remove excess moisture. An aliquot of the sample (1.0ml) was taken and evaporated to dryness at 60°C. The residue was dissolved in 1.0 mL of chloroform. Standards (vitamin A palmitate) of concentrations ranging from 0-7.5 g were pipetted out into a series of test tubes. The volume in all the tubes was made up to 1.0ml with chloroform. TCA reagent (2.0ml) was added rapidly, mixed, and the absorbance was read immediately at 620nm in a spectrophotometer (Genesys 10UV). The same procedure was repeated for the sample tubes also. Vitamin A content was expressed as mg/l.

3.2.8.2 Vitamin E estimation

Vitamin E was estimated in the samples by the Emmerie-Engel reaction as reported by Rosenberg (1992). The sample (2.5g) was homogenised in 50ml of 0.1N sulphuric acid and allowed to stand overnight. The contents of the flask were shaken vigorously and filtered through Whatman No.1 filter paper. Aliquots of the filtrate were used for the estimation. Into 3 stoppered centrifuge tubes, 1.5ml of sample, 1.5ml of the standard and 1.5ml of water were pipetted out separately. To all the tubes,

1.5ml of ethanol and 1.5ml of xylene were added, mixed well and centrifuged. Xylene (1.0ml) layer was transferred into another stoppered tube. To each tube, 1.0ml of dipyridyl reagent was added and mixed well. The mixture (1.5ml) was pipetted out into a cuvette and the extinction was read at 460nm.

3.2.8.3 Vitamin C estimation

Vitamin C was analysed by the spectrophotometric method described by Roe and Keuther (1943). Ascorbate was extracted from 1g of the sample using 4% TCA and the volume was made up to 10ml with the same. The supernatant obtained after centrifuging at 2000rpm for 10minutes was treated with a pinch of activated charcoal, shaken vigorously using a cyclomixer and kept for 5 minutes. The charcoal particles were removed by centrifugation and aliquots were used for the estimation. Standard ascorbate ranging between 0.2 – 1.0ml and 0.5ml and 1.0ml of the supernatant were taken. The volume was made up to 2.0ml with 4% TCA. DNPH reagent (0.5ml) was added to all the tubes, followed by 2 drops of 10% thiourea solution. The contents were mixed and incubated at 37⁰c for 3 hours resulting in the formation of osazane crystals. The crystals were dissolved in 2.5ml of 85% sulphuric acid, in cold. To the blank alone, DNPH reagent and thiourea were added after the addition of sulphuric acid. The tubes were cooled in ice and the absorbance was read at 540nm in a spectrophotometer. A standard graph was constructed using an electronic calculator set to the linear regression mode. The concentration of ascorbate in the sample were calculated and expressed in terms of mg/kg of sample.

3.2.8.4 Determination of Vitamin B₁ and B₂

Vitamins B₁ and B₂ was analysed by the spectrophotometric method described by Roe and Keuther (1943). One gram (1g) of sample was weighed into a conical flask. This was dissolved in 100ml of deionised water, shaken thoroughly, heated for 5 minutes, allowed to cool, and then filtered. The filtrate was poured into cuvette and their respective wavelengths (261nm, and 242nm) for vitamins B₁ and B₂ set to read the absorbance using a spectrophotometer.

Calculations:

$$\text{concentration (mg\%)} = \frac{A \times DF \times \text{volume of cuvette}}{E} \quad 3.7$$

Where A = absorbance

E = extinction coefficient = 25 for B1 and B

DF = dilution factor

3.2.8.5 Determination of Vitamin B₃ (Nicotinamide)

Five gram (5g) of sample was dissolved in 20ml of anhydrous glacial acetic acid and warmed slightly. 5ml of acetic anhydride was added and mixed. 2 -3 drops of crystal violet solution were added as an indicator. The mixture was titrated with 0.1M perchloric acid to a greenish blue colour.

Calculation:

$$\text{Vitamin B}_3 = \frac{\text{titre value} \times 0.0122}{0.1} \quad 3.7$$

3.2.8.6 Determination of Vitamin B₆

Five gram (5g) of sample was dissolved in a mixture of 5ml of anhydrous glacial acetic acid and 6ml of 0.1m mercury II acetate solution. 2 drops of crystal violet was added as indicator. The mixture was titrate with 0.1m perchloric acid to a green colour end point.

3.2.8.7 Estimation of Vitamin B₁₂

Spectrophotometric determination of cyanocobalamine in serum preparations by coupling reactions with pyridine

Equivalent 0.1ml of sample was weighed, and taken into separator. In separator, 5 ml of water was added, mixed well and extracted with 5 ml chloroform. The water layer was discarded then chloroform layer was taken in dry 50 ml volumetric flask by passing through anhydrous sodium sulphate and

made up to 50 ml with chloroform. 2ml sample and blank solution was taken into test tube. In each test tube, 2 ml of 0.2% solution of phenyl hydrazine (in hydrochloric acid and alcohol in ratio of 1:5 v/v) was added, and mixed well. It was then heated over a water bath until to almost dryness and cool at room temperature. 2ml solution mixture (ammonia and alcohol in ratio of 1:1) was added in each test tube and 1ml pyridine was. Its absorbance recorded at 635 nm against blank. Standard cobalamine was also analyzed and treated same as sample. Calibration curve was plotted and the concentration of sample extrapolated. (Hasfat 2010).

3.2.8.8 Vitamin D estimation

Vitamin D was assayed according to the method of Brockmann et al. (1974). 25 mg vitamin D₃ working standard was taken into a 25 ml volumetric flask with solution mixture (chloroform and methanol in ratio of 1:9). It was dissolved and diluted with solution mixture and made up to the mark, and mixed well. Equivalent 0.1ml sample was accurately weighed into a 25 ml volumetric flask with solution mixture (chloroform and methanol in ratio 1:9) dissolved and dilute with solution mixture and make up to the mark, and mixed well. 1.6 ml of 0.25N HCL, 0.5ml of 15.0% trichloroacetic acid (TCA) and 0.5ml of 0.375% of thiobarbituric acid (TBA) was added. Its absorbance was recorded at 464 nm against blank.

3.2.9 Mineral Analysis

Mineral analysis was conducted using Agilent FS240AA Atomic Absorption Spectrophotometer according to the method of APHA 1995 (American Public Health Association). Approximately 2g of the dried sample was weighed out in to a digestion flask, and 20ml of the acid mixture (650ml conc HNO₃; 80ml perchloric acid; 20ml conc H₂SO₄) was added. The flask was heated until a clear digest is obtained. The digest was diluted with distilled water to the 100ml mark.

3.2.9.1 Preparation of Reference Solutions

A series of standard metal solutions in the optimum concentration range are prepared, the reference solutions were prepared daily by diluting the single stock element solutions with water containing 1.5 mL concentrated nitric acid/litre. A calibration blank was prepared using all the reagents except for the metal stock solutions.

Calibration curve for each metal.

3.2.10. In Vitro Antioxidant Activity

3.2.10.1 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

The scavenging ability of the natural antioxidants of the plant samples towards the stable free radical DPPH was measured by the method of Mensor et al. (2001). 20µl of the sample was added to 0.5ml of 0.1mM methanolic solution of DPPH and 0.48ml of methanol. The mixture was allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol, without the leaf samples, served as the positive control while butylated hydroxytoluene (BHT) served as reference. After 30 minutes of incubation, the discolouration of the purple colour was measured at 518nm in a spectrophotometer (Genesys 10-S, USA). The radical scavenging activity was calculated as follows:

$$\text{Scavenging activity \%} = 100 - \frac{A_{518}(\text{sample}) - A_{518}(\text{blank})}{A_{518}(\text{blank})} \times 100$$

A518 (blank)

3.9

3.2.10.2 Ferric Reducing Antioxidant Property (FRAP)

The reducing property of the extracts was determined as described by Pulido et al. (2000). 0.25 ml of the extracts will be mixed with 0.25 ml of 200 mM Sodium phosphate buffer pH 6.6 and 0.25 ml of 1% Potassium ferrocyanide. The mixture will be incubated at 50oC for 20 min, thereafter 0.25 ml of

10% trichloroacetic acid will be added and centrifuge at 2000 rpm for 10 min, 1 ml of the supernatant will be mixed with 1 ml of distilled water and 0.2 ml of ferric chloride and the absorbance was measured at 700 nm.

3.2.10.3 Hydrogen Peroxide Scavenging Activity

The ability of the samples to scavenge hydrogen peroxide was assessed by the method of Ruch et al. (1989). A solution of H₂O₂ (40mM) was prepared in phosphate buffer. The sample at the concentration of 10mg/10μl was added to H₂O₂ solution (0.6ml) and the total volume was made up to 3ml. The absorbance of the reaction mixture was recorded at 230nm in a spectrophotometer (Genesys 10-S, USA). A blank solution containing phosphate buffer, without H₂O₂ was prepared. The extent of H₂O₂ scavenging of the sample samples was calculated as

$$\% \text{scavenging activity} = \frac{A_0 - A_1}{A_0} \times 100 \quad 3.10$$

A₀ - Absorbance of control

A₁ - Absorbance in the presence of sample.

3.2.10.4 Superoxide Scavenging Activity

The superoxide scavenging ability of the samples was assessed by the method of Winterbourn, Hawkins, Brian, & Carrell (1975). Superoxide anions were generated in samples that contained in 3.0ml, 0.02ml of the leaf samples (20mg), 0.2ml of EDTA, 0.1ml of NBT, 0.05ml of riboflavin and 2.64ml phosphate buffer. The control tubes were also set up where DMSO was added instead of the sample. All the tubes were vortexed and the initial optical density was measured at 560nm in a spectrophotometer (Genesys, 10-S, USA). The tubes were illuminated using a fluorescent lamp for 30 minutes. The absorbance was measured again at 560nm. The difference in absorbance before and after illumination was indicative of superoxide anion scavenging activity.

3.2.10.5 Nitric Oxide Scavenging Activity

The extent of inhibition of nitric oxide radical generation *in vitro* was followed as per the method reported by Green et al. (1982). The reaction was initiated by adding 2.0ml of sodium nitroprusside, 0.5ml of PBS, 0.5ml of sample (50mg) and incubated at 25°C for 30 minutes. Griess reagent (0.5ml) was added and incubated for another 30 minutes. Control tubes were prepared without the samples. The absorbance was read at 546nm against the reagent blank, in a spectrophotometer (Genesys 10-S, USA).

3.2.10.6 Hydroxyl Radical Scavenging Activity

The extent of hydroxyl radical scavenging from Fenton reaction was quantified using 2'-deoxyribose oxidative degradation as described by Elizabeth and Rao (1990). The reaction mixture contained 0.1ml of deoxyribose, 0.1ml of FeCl₃, 0.1ml of EDTA, 0.1ml of H₂O₂, 0.1ml of ascorbate, 0.1ml of KH₂PO₄-KOH buffer and 20 µl of sample in a final volume of 1.0ml. The mixture was incubated at 37°C for 1 hour. At the end of the incubation period, 1.0 ml of TBA was added and heated at 95°C for 20 minutes to develop the colour. After cooling, the TBARS formation was measured spectrophotometrically (Genesys 10-S, USA) at 532nm against an appropriate blank. The hydroxyl radical scavenging activity was determined by comparing the absorbance of the control with that of the samples. The percent TBARS production for positive control (H₂O₂) was fixed at 100% and the relative percent TBARS was calculated for the sample treated groups.

3.2.10.7 Assay of Malondialdehyde

The extent of MDA was estimated according to the method of Okhawa et al. (1979). A 20% liver homogenate was prepared in phosphate buffer (pH 7.2). To 0.5ml of the homogenate, 1.0ml of TCA and 1.0ml of TBA were added and mixed thoroughly. The mixture was heated in a boiling water bath for 20 minutes. The tubes were centrifuged at 1000g for 10 minutes and the absorbance was read at

535nm in a spectrophotometer (Genesys 10-S, USA) against a blank containing all the reagents except the homogenate. The MDA equivalents of the samples were calculated using the extinction coefficient $1.56 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$

3.2.10.8 Assay of Superoxide Dismutase (SOD)

SOD was assayed according to the method of Kakkar et al. (1984). The different samples, (1.0g), were ground with 3.0ml of potassium phosphate buffer, centrifuged at 2000g for 10 minutes and the supernatants were used for the assay. The assay mixture contained 1.2ml of sodium pyrophosphate buffer, 0.1ml of PMS, 0.3ml of NBT, 0.2ml of the enzyme preparation and water in a total volume of 2.8ml. The reaction was initiated by the addition of 0.2ml of NADH. The mixture was incubated at 30°C for 90 mins and arrested by the addition of 1.0ml of glacial acetic acid. The reaction mixture was then shaken with 4.0ml of n-butanol, allowed to stand for 10 minutes and centrifuged. The intensity of the chromogen in the butanol layer was measured at 560nm in a spectrophotometer (Genesys 10-S, USA).

3.2.10.9 Catalase (CAT) Assay

Catalase activity was assayed following the method of Luck (1974). A 20% homogenate of the Sample was prepared in phosphate buffer. The homogenate was centrifuged and the supernatant was used for the enzyme assay. H_2O_2 -phosphate buffer (3.0ml) was taken in an experimental cuvette, followed by the rapid addition of 40 μl of enzyme sample and mixed thoroughly. The time required for a decrease in absorbance by 0.05 units was recorded at 240nm in a spectrophotometer (GAERenesys 10-S, USA). The enzyme solution containing H_2O_2 -free phosphate buffer served as control.

3.2.10.10 Estimation of Reduced Glutathione

Reduced glutathione was determined by the method of Moron et al. (1979). A homogenate was prepared with 0.5g of the sample with 2.5ml of 5% TCA. The precipitated protein was centrifuged at

1000 rpm for 10 minutes. The supernatant (0.1ml) was used for the estimation of GSH. The supernatant (0.1ml) was made up to 1.0ml with 0.2M sodium phosphate buffer (pH 8.0). Standard GSH corresponding to concentrations ranging between 2 and 10 nmoles were also prepared. Two ml of freshly prepared DTNB solution was added and the intensity of the yellow colour developed was measured in a spectrophotometer (Genesys 10-S, USA) at 412nm after 10 minutes. The values are expressed as nmoles GSH/g sample.

3.2.10.11. Estimation of Total Phenols

The amount of total phenols in the sample tissues was estimated by the method proposed by Malick and Singh (1980). The sample (0.5g) was homogenized in 10X volume of 80% ethanol. The homogenate was centrifuged at 10,000rpm for 20 minutes. The sample ion was repeated with 80% ethanol. The supernatants were pooled and evaporated to dryness. The residue was then dissolved in a known volume of distilled water. Different aliquots were pipette out and the volume in each tube was made up to 3.0ml with distilled water. Folin- Ciocalteau reagent (0.5ml) was added and the tubes were placed in a boiling water bath for exactly one minute. The tubes were cooled and the absorbance was read at 650nm in a spectrophotometer (Genesys 10-S, USA) against a reagent blank. Standard catechol solutions (0.2-1ml) corresponding to 2.0-10 μ g concentrations were also treated as above. The concentration of phenols is expressed as mg/g tissue.

3.2.10.12 Total Antioxidant Capacity (TAC) assay:

The Total Antioxidant Capacity (TAC) of extract was determined by the phosphomolybdate method according to Jayaprakasha et al. (2002). An aliquot (30 mL) of different concentrations (20, 40, 60, 80 and 100 mg mL⁻¹) of the test extracts was mixed with 3 mL of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, 4 mM ammonium molybdate) taken in test tubes. The tubes were capped with aluminium foil and incubated in a boiling water bath at 95°C for 90 min. The

reaction mixture was allowed to cool to room temperature and the absorbance of the solution was measured at 695 nm against a blank containing 3 mL of reagent solution and the appropriate volume of the dissolving solvents. The blank was incubated under the same conditions as the test samples. Ascorbic acid was used as standard reference compounds to compare the activities of the extracts.

3.2.11 *In vivo* Study

3.2.11.1 Procurement of animals

For this study, adult male Wistar rats weighing 100-150 g were used. The animals were procured from the Animal Breeding Unit , Zoology Department , University of Nigeria, Nsukka (UNN) , Enugu State, Nigeria and were acclimatised in an airy pen at room temperature, fed with standard rat feed, and given water *ad libitum* for two weeks. The NIH recommendations for the care and use of laboratory animals were followed in the experimental procedures.

3.2.11.2 Acute Toxicity Study (LD₅₀)

The analysis was carried out using Lorke's method (1983) to determine the acute lethal dose/toxicity of the leaves, stem bark, and pulp.

Phase 1: A total of 27 albino mice were used. The experimental design contained 9 groups of 3 mice each . They were administered the extracts in low doses of 10 mg/kg, 100 mg/kg, and 1000 mg/kg body weight of each plant part ethanolic extract sequentially for one week. The animals were observed for 24 hours for signs such as paw licking, salivation, stretching of entire body, weakness, respiratory distress, coma and mortality within the first 4 hours and subsequently for 14 days.

Phase 2: A total of 18 albino mice were used. The experimental design contained 9 groups of 2 mice each . They were administered the extracts in high doses of 3000mg/kg, 4000mg/kg, and 5000mg/kg

body weight sequentially for one week, and were observed for 24 hours for signs of toxicity and mortality within the first 4 hours and subsequently for 14 days.

3.2.12 Experimental Design

After the 14 days acclimatization period, the animals was randomly divided into 11 groups of 5 rats each;

Table 3.2: Benign Prostatic Hyperplasia (BPH) study design

Groups	Group label	Number of Rats	Treatment	Duration of treatment
1	Normal control	5	Feed and water	21 days
2	Negative control	5	Induced with BPH (5.0 mg/kg per b.w testosterone propionate)	21 days
3	Standard control	5	BPH-induced and treated with a standard drug (0.5mg/kg b.w finasteride)	21 days
4	Test group	5	Induced and treated with 250mg/kg body weight of plant leaf extract	21 days
5	Test group	5	Induced and treated with 500mg/kg body weight of plant leaf extract	21 days
6	Test group	5	Induced and treated with 250mg/kg body weight plant stem bark extract	21 days
7	Test group	5	Induced and treated with 500mg/kg body weight of plant stem bark extract.	21 days
8	Test group	5	Induced and treated with 250mg/kg body weight of plant pulp extract	21 days
9	Test group	5	Induced and treated with 500mg/kg body weight of plant pulp extract	21 days

10	Test group	5	Induced and treated with 250mg/kg body weight of plant leaves, pulp and stem bark extracts	21 days
11	Test group	5	Induced and treated with 500mg/kg body weight of plant leaves, pulp and stem bark extracts	21 days

3.2.12.1 BPH Induction and Treatments

Benign prostatic hyperplasia was induced in albino rats using testosterone propionate (5.0 mg/kg per b.w) with olive oil as the vehicle for 21 days as carried out by Ekeyi et al. (2021) with modifications, followed by oral treatment with 0.5mg/kg b.w of Finasteride (group 3) adapted from Alamoudi et al. (2022), and plant extracts at different doses for another 21 days, and monitored for changes.

3.2.12.2 Body Weight and Organ Weight Measurements

The body weights of the rats were taken weekly while their organ weights were taken at the end of the experiment (after sacrifice) using a Top loader weighing balance.

3.2.12.3 Sacrifice of Animals

At the end of the treatment period, all rats were fasted overnight, sacrificed under mild anaesthesia using chloroform and blood samples were collected through cardiac puncture into heparin and non-heparin bottles for biochemical analysis. Prostate, liver and kidneys were excised, stored in 10% formal saline and transported to the laboratory for histological examination.

3.2.13 Biochemical Assay

3.2.13.1 Hematological studies

Haematological analysis of the blood samples was performed in an automated haematology analyzer (EMA D-6031 model, Bioevopeak, China) with the procedure carried as specified by the producer.

The parameters which were evaluated included: red blood cells count(RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV); mean corpuscular haemoglobin (MCH); mean corpuscular haemoglobin concentration (MCHC); platelets (PLT); total leukocytes count (TLC) count and differential leukocytes count(WBC) counts were obtained at once for each blood sample. These parameters were analysed using modified automated Wallace H. Coulter method.

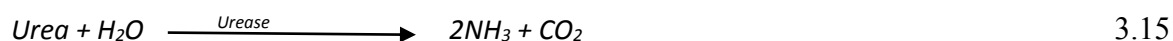
3.2.13.2 Kidney function tests

3.2.13.2.1 Determination of serum urea

Urea concentration in the sample was carried out according to the method described by Searcy, Reardon & Foreman (1967).

Principle

Urea in serum is hydrolysed to ammonia in the presence of urease. The ammonia is then measured photometrically by Berthelot's reaction.



Procedure

Three test tubes labelled T, S and B for test, standard and blank respectively were set up. And the following procedures listed below were taken.

To test tube T, pipette 10µl of serum, to test tube S, pipette 10µl of creatinine standard and to test tube B, pipette 10µl of distilled water. Add 100 µl of R1 reagent to all test tubes then mix well and incubate at 37°C for 10 mins. Then add 2.5ml of R2 to all test tubes followed by the addition of 2.5ml of R3 to

all test tubes. Mix the content of the test tubes and incubate at 37°C for 15 mins. Read the absorbance of test and standard against the blank.

$$\text{Calculation: Urea conc. (mmol/l)} = \frac{A_{\text{test}}}{A_{\text{standard}}} \times \text{conc. of standard (12.70mmol/l)} \quad 3.17$$

3.2.13.2.2 Determination of serum creatinine

Serum creatinine concentration in the sample was determined according to the method described by Toora & Rajagopa (2002).

Principle; Creatinine in alkaline medium reacts with picrate to form a coloured complex whose colour intensity is directly proportional to the creatinine concentration in serum at 520nm



Procedure

Label two test tubes ‘sample’ and ‘standard’

Pipette 200µl of creatinine standard into the test tube labelled standard and 200µl of serum sample into test tube labelled sample test tube then 200µl of working reagent was added to the two test tubes.

The content of the individual tubes were mixed and a stopwatch started immediately. After 30 seconds, the absorbance A_1 was read at 492nm. The absorbance A_2 was read again after exactly 2 mins. $A_2 - A_1 = A_{\text{sample}}$ or A_{standard} as the case may be. The mixtures were allowed to stand for 20 minutes at 25 °C and the absorbance of the sample and standards read against the blank at 540 nm.

Calculation:

$$\text{creatinine concentration} = \frac{A_1}{A_2} \times (\text{concentration of standard}) \quad 3.19$$

3.2.13.3 Determination of serum electrolyte

3.2.13.3.1 Serum sodium concentration

Serum sodium concentration was estimated using commercial kits and following standard protocols prescribed by the producer according to the method described by Maruna (1958) and Trinder (1951) with modifications. Three test tubes properly labelled test, standard and blank were set up and 1.0 ml of filtrate reagent was pipetted into each test tube. Exactly 50µl of the sample was added to the test tube labelled test and the same volume of the standard reagent to the standard and the same volume of distilled water to the blank. All test tubes were shaken vigorously to ensure adequate mixing of their contents. Each test tube was centrifuged at high speed for 10 minutes to obtain a supernatant above and precipitate below. Care was taken not to disturb the protein precipitate. The test proceeded to the stage with the supernatant only. Another set of three test tubes still labelled test, standard and blank were set up and 1 ml of the acid reagent was pipetted into each of the tubes. Then 50µl of the supernatants was added to their corresponding test tubes and mixed properly. Also, 50µl of the colour reagent was added to all the tubes and mixed. The spectrophotometer was zeroed with distilled water and then the absorbance of the content of each test tube was read and recorded. The concentration of sodium in the sample was calculated using the expression:

$$\text{Sodium conc. in mEq/L} = \frac{\text{Abs. of blank} - \text{Abs. of test}}{\text{Abs. of blank} - \text{Abs. of standard}} \times \text{Conc. of standard} \quad 3.20$$

Where concentration of standard = 150 mEq/L

3.2.13.3.2 Serum chloride concentration

Serum chloride concentration was estimated using commercial kits and following standard protocols prescribed by the producer according to method described by Skeggs and Hochstrasser (1964). Three test tubes properly labeled test, standard and blank were set up and 1.5ml of the chloride reagent was

pipetted into each tube. Then 10µl of the sample was added to the test tube labeled test and the same volume of the standard reagent to the standard and the same volume of distilled water to the blank. All test tubes were shaken to mix and incubated at 25⁰C for 5 minutes. Then the absorbance of the content of each test tube was read at wavelength 500 nm after zeroing with the blank.

Calculate chloride concentration in mEq/L with the expression:

$$Cl^- \text{ concentration in mEq/L} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{concentration of standard} \quad 3.21$$

Where concentration of standard = 100 mEq/L

3.2.13.3 Serum bicarbonate concentration

Serum bicarbonate concentration was estimated using commercial kits and following standard protocols prescribed by the producer according to the method of Kaplan et al., (1984). Carbon dioxide (CO₂) reagent was reconstituted with the volume of CO₂ free water indicated on the vial label, mixed by gentle inversion 5-6 times. Three cuvettes labelled test, standard and blank were set up and 1 ml of the reagent was pipetted into each tube and all tubes were incubated for 3 minutes at 37⁰C. Exactly 10µl of the sample were added to the cuvette labelled test and the same volume of the standard reagent and distilled water to the standard and blank respectively. The absorbance was read at 340 nm and the bicarbonate concentration calculated thus:

$$\text{Bicarbonate conc. in mmol/L} = \frac{\text{Abs of blank} - \text{Abs of test}}{\text{Abs of blank} - \text{Abs of standard}} \times \text{Conc. of standard} \quad 3.22$$

Where concentration of standard = 30 mmol/L

3.2.13.4. Liver Function Tests

3.2.13.4.1 Determination of serum alkaline phosphatase

The serum activity of alkaline phosphatase (ALP) was determined using Randox test kits.

Principle

p-Nitrophenylphosphate is hydrolysed in the presence of magnesium ions by phosphatase to phosphate and p-nitrophenol. The p-nitrophenol liberation is proportional to the alkaline phosphatase activity and can be measured photometrically. The standard colorimetric method is according to the recommendation of the Deutsche Gesellschaft für Klinische Chemie.



Procedure

To 0.02ml of the serum sample in a cuvette was added 1.0ml of Randox ALP reagent. The content was mixed and the initial absorbance was taken, after which the readings were taken after 1, 2 and 3 mins, at 405nm in a spectrophotometer.

Calculations:

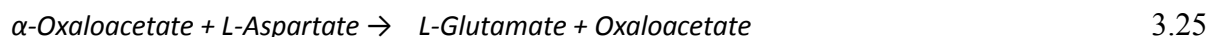
$$\text{The ALP activity of the serum (U/L)} = 2760 \times \Delta A_{405\text{nm}}/\text{min} \quad 3.24$$

3.2.13.4.2 Determination of serum aspartate transaminase activity

The serum activity of aspartate transaminase (AST) or glutamate oxaloacetate transaminase (GOT) was determined using Randox test kits.

Principle

AST was measured by monitoring the concentration of oxaloacetate hydrazine formed with 2,4-dinitrophenylhydrazine.



Procedure

Two test tubes were set up labeled T₁ (reagent blank) T₂ (test sample). T₁ contained 0.10ml distilled water and 0.05ml Randox buffer solution, while T₂ contained 0.10ml serum sample and 0.50ml

Randox buffer solution. The contents were mixed and incubated for 30mins at 37°C. To each test tube was added 0.50ml of Randox 4-dinitrophenylhydrazine solution and the contents were mixed and allowed to stand for 20mins at 25°C. Then 5ml of the 0.4M NaOH solution was added to each of the tubes. The contents were mixed, and after 5mins, their absorbances were read at 546nm against the reagent blank in a spectrophotometer.

Calculation

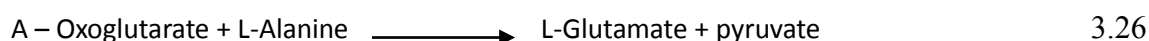
The AST activity was obtained by comparison to the table provided in the kits leaflet,

3.2.13.4.3 Determination of serum alanine transaminase activity

The serum activity of alanine transaminase (ALT) or glutamate pyruvate transaminase (GPT) was determined using Randox test kits (Randox Laboratories, Crumlin, England).

Principle

ALT was measured by monitoring the concentration of pyruvate hydrazone formed with 2,4 – dinitrophenylhydrazine



Procedures

Two test tubes were set up T₁ (reagent blank) and T₂ (test sample). T₁ contained 0.10mL distilled water and 0.50mL randox buffer solution, while T₂ contained 0.10mL serum sample and 0.50mL Randox buffer solution. The contents were mixed and incubated for 30min at 37°C. To each test tube was added 0.50mL of Randox 4 – dinitrophenylhydrazine solution and the contents were mixed and allowed to stand for 20min at 25°C. Then 5mL of sodium hydroxide solution was added to each of the tubes. The contents were mixed and after 5min, their absorbance were read at 546nm against blank in a spectrophotometer Model 721 (spectrumlab).

Calculation

The ALT activity was obtained by comparison to the table provided in the kit's leaflet as shown in appendix

3.2.13.4.4 Serum total bilirubin content:

Serum bilirubin content was estimated using commercial kits (Randox, UK) and following standard protocols according to the Colorimetric method based on that described by Jendrassik and Grof (1938).

Principle

Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin, by the reaction with diazotised sulphanilic acid.

Procedure

Two test tubes were set up and labeled blank and sample. To the sample test tube, 200µl of Reagent 1 (0.17N hydrochloric acid), 50µl of Reagent 2 (38.5mmol/L of sodium Nitrite), 1000µl of Reagent 3 (0.52mmol/L sodium benzoate) and 200µl of serum were added and mixed properly while only 200µl of Reagent 1 and 1000µl of reagent R3 were added to the blank test tube. The 2 test tubes were then incubated at 20-25°C for 10 minutes, after which 1000µl of reagent 4 (1.9N sodium Hydroxide) was added to both test tubes. The test tubes were incubated for a further 30 minutes at 25°C before reading absorbance on a spectrophotometer at 546nm after zeroing with blank. To obtain total bilirubin in mg/dl the formular below was used.

$$\text{Total Bilirubin} = 10.8 \times \text{Absorbance of Sample} \quad 3.27$$

3.2.13.4.5 Serum direct bilirubin content:

Serum bilirubin content was estimated using commercial kits (Randox, UK)

Principle

Direct (conjugated) bilirubin reacts with diazotised sulphanilic acid in alkaline medium to form a blue coloured complex.

Procedure

Two test tubes were set up and labeled blank and sample. To the sample test tube, 200µl of Reagent 1 (0.17N hydrochloric acid), 50µl of Reagent 2 (38.5mmol/L of sodium Nitrite), 2000µl of 0.9% NaCl and 200µl of serum sample were added and mixed properly while only 200µl of Reagent 1 and 2000µl of 0.9% NaCl were added to the blank test tube. The two test tubes were then incubated at 20-25°C for 10 minutes, before reading absorbance on a spectrophotometer at 546nm after zeroing with blank. To obtain direct bilirubin in mg/dl the formular below was used.

$$\text{Direct Bilirubin (mg/dl)} = 14.4 \times \text{Absorbance of Sample} \quad 3.28$$

3.2.13.4.5 Determination of total protein concentration((Lubran, 1978)

Principle: Cupric ions interact with protein peptide bonds resulting in the formation of colored complex.

Procedure: Total protein concentration of the samples was determined using kit obtained from Randox Diagnostics Ltd, USA. An aliquot (0.01 ml) of distilled Water (reagent blank), standard, sample blank and serum samples were dispensed into their respective test tubes and 0.5 ml of Biuret reagent (containing 100, 16, 15 and 6 mmol/l of NaOH, Na-K-tartrate, potassium iodide and cupric sulphate, respectively) was added to all test tubes and mixed. The reaction mixture was incubated for 30 min at room temperature and read at 540 nm

$$\text{Total protein concentration (g/l)} = \frac{\text{Absorbance of sample} \times \text{standard conc (g/l)}}{\text{Absorbance of Standard}} \quad 3.29$$

3.2.13.4.6 Determination of albumin concentration

This was carried out using the method of Grant, (1987)

Principle: This assay is based on the quantitative binding of serum albumin to the indicator 3,3',5,5'-tetrabromo-m cresol sulphonephthalein (bromocresol green, BCG) to form a complex which absorbs at 630 nm (Grant, 1987).

Procedure: The products of Randox Diagnostics Ltd, USA were used. Aliquots (0.01 ml) of distilled water (reagent blank), standard and serum samples were dispensed into their appropriately labeled test tubes and mixed with 3.0 ml of BCG reagent. The reaction mixture was incubated at room temperature for 5 min and read at 630 nm. Absorbance of the sample and standard were measured against the reagent blank.

$$\text{Albumin concentration (g/l)} = \frac{\text{Absorbance of sample} \times \text{standard conc (g/l)}}{\text{Absorbance of Standard}} \quad 3.30$$

3.2.13.7 Estimation of serum globulin

According to the method of George , (2009)

Since, bromocresol green (BCG) – albumin complex absorbs light at a different wavelength from the unbound dye, the method may overestimate albumin by binding to other proteins (George, 2009). Hence, the total globulin fraction is generally determined by subtracting the albumin fraction from the total protein fraction.

3.2.14. Determination of Prostate Biomarkers

3.2.14.1 Determination of TNF- α Using enzyme-linked immunosorbent assay (ELISA)

The procedure for quantifying the concentration of TNF- α in cellular medium was carried out according to the instructions of the ELISA kit (R&D Systems; Minneapolis, USA). Briefly, 100- μ L samples were added to the wells of a 96-well ELISA plate coated with anti-TNF- α monoclonal antibodies and mixed well. Blank control wells were set up and processed at the same time. The plate was sealed and then incubated at 37 °C for 90 min. The plate was washed four times with washing buffer. 100 μ L diluted biotinylated antibody was added to each well and mixed thoroughly. The plate was sealed and incubated at 37 °C for 60 min. After washing for four times with washing buffer, a HRP-labeled avidin (100 μ l) was added to each well and mixed. The plate was incubated at 37 °C for 30 min after being sealed. After washing, a colored liquid (100 μ L) was added to each well and the plate was incubated at 37 °C in the dark for 15 min before 100 μ L stop buffer was added to each well and mixed to stop the reaction. The absorbance values of each well were measured at 450 nm as the optical densities (ODs). The OD values were used as ordinates, with the standard concentrations on the x-axis and the absorbance values on the y-axis, and a standard curve was drawn for these data. The sample concentrations were calculated according to the OD values of the samples based on the standard curve. Triplicate wells were set up for each group for statistical analysis. At least three independent experiments were repeated.

3.2.14.2 Determination of prostate specific antigen (PSA) level

The PSA random kit is based on a solid phase sandwich enzyme-linked immunosorbent assay. The assay system utilizes an anti-PSA monoclonal antibody for solid phase (microtiter wells) immobilization and another anti-PSA monoclonal antibody as antibody-enzyme (horseradish peroxidase) conjugate reagent. PSA in the reference standards or in the patient's specimens binds to anti-PSA on the well and the anti-PSA second antibody then binds to PSA. Unbound protein and horse raddish peroxidase conjugate are removed by washing. Upon the addition of the substrate, the horseradish peroxidase activity bound on

the wells is then assayed by a chemi-luminescence reaction. The related light unit (RLU) of the reaction is proportional to the concentration of PSA presented in the specimen.

Procedure

In the desired number of coated wells secured in a holder, 25µl of PSA standards was added to the specimens, and controls into the appropriate wells, and then 100µl of Enzyme Conjugate Reagent was added also into each well and mixed thoroughly for 30 seconds and incubated at 37°C for 60 minutes. The incubation mixture was removed by flicking plate contents into a waste container. The microtiter plate was rinsed and flicked 5 times with distilled water. The plate was struck sharply onto absorbent paper to remove residual water droplets. 50µl of Substrate A and 50µl of Substrate B was added into each well and mixed gently for 10 seconds. The microplate was then put into the detecting chamber of a luminometer for 5 minutes, and then values of each well was read.

3.2.14.3 Determination of testosterone level

The samples were assayed in batches from a standardized curve using the enzyme linked immunosorbent assay (ELISA) method. The microwell kits used were from Syntro Bioresearch Inc., California USA. Using 10 µl of the standard, the samples and control were dispensed into coated wells. 100 µl T conjugate reagent was added followed by 50 µl of anti-T reagent. The contents of the microwell were thoroughly mixed and then incubated for 90 min at room temperature. The mixture was washed in distilled water and further incubated for 20 min. The reaction was stopped with 100 µl of 1N hydrochloric acid. Absorbance was measured with an automatic spectrophotometer at 450 nm. A standard curve was obtained by plotting the concentration of the standard versus the absorbance and T concentration was determined from the standard curve.

3.2.15 Determination of acid phosphatase (ACP) activity (Randox kit)

1.0ml of sample (homogenate) was measured into a test tube and 0.5ml of reagent 1 (sodium citrate pH 5.2) was added. The mixture was equilibrated in a water bath for 10 minutes at 37°C. 0.5ml of reagent 2 (α -naphthyl-phosphatase) was added. The mixture was incubated for five minutes. The initial absorbance at 450nm was read at one-minute intervals thereafter for 3 minutes. The difference between the absorbance and average absorbance differences per minute ($\Delta A/\text{min}$) was then estimated.

The enzyme activity was calculated using

$$\Delta A/\text{min} \times 743 \qquad \qquad \qquad 3.31$$

3.2.15.1 Preparation of liver

One gramme (1g) of the liver tissues were homogenized in 10ml of ice-cold physiological saline to obtain 10% (w/v) homogenates. The resulting homogenates were centrifuged at 4,000 rpm for 10min and the supernatants obtained were used for determination of superoxide dismutase (SOD), catalase (CAT) and lipid peroxidation level.

3.2.15.2 Determination of Antioxidant Parameters

3.2.15.2.1 Determination of malondialdehyde (Slater & Sawyer, 1971)

This was done using the method of Slater and sawyer, (1971). Malondialdehyde in plasma is one of the aldehyde products of lipid peroxidation which react with TBA to form a colored product, the absorbance of which is measured spectro-photometrically at 530 nm. In the test tubes 0.5 ml of hemogenate from test samples were taken, and 3 ml of 10% TCA was added to it, mixed well and the tubes were left to stand for 10 min at room temperature, and then centrifuged for 15 min at 4000 rpm. Two sets of test tubes were taken marked as blank and test. For a test sample, 2 ml of supernatant

fluid was taken and added to 1.5 ml of 0.67% TBA. For a blank sample, 2 ml of distilled was added in 2 ml of 0.67% TBA. After mixing well and keeping in the boiling water bath for 10 min, they were cooled to room temperature. A pale pink color developed, the color intensity was measured at 530 nm by spectro-photometer. Using the molar extension coefficient (1.5×10^5) and result was expressed as n moles of malonaldehyde (MDA)/100 ml of hemogenate.

$1.5 = 100 \mu\text{mol/L}$ (here, 100 is for conversion from ml to dl).

Then $MDA = (100 \times \text{Absorbance of unknown}) / (1.5)$ 3.34

3.2.15.2.2 Superoxide dismutase assay

The method described by kakkar et al., (1984) was used in the SOD determination

This was estimated by the reaction mixture which contained 0.1 mL of phenazine methosulfate (186 μM), 1.2 mL of sodium pyrophosphate buffer (pH 7.0), 0.3 mL of the supernatant after centrifugation of homogenate was added to the reaction mixture. Enzyme reaction was initiated by adding 0.2 mL of NADH (780 μM) and stopped after 1 min by adding 1 mL of glacial acetic acid. The amount of chromogen formed was measured by recording color intensity at 560 nm. Results were expressed in units/mg protein.

3.2.15.2.3 Estimation reduced glutathione (GSH)

The method of Jollow et al. (1974) was followed to measure GSH activity in the liver homogenate. Briefly, 500 μl of supernatant was mixed with 500 μl of sulfosalicylic (4%) to carry out precipitation. The reaction mixture was incubated for 1 h at 4 °C and then centrifuged at 4000 rpm for 20 min. Supernatant was collected and 33 μl of it was added to the reaction mixture containing 66 μl of 100 mM of 5,5'-dithio-bis (2-nitrobenzoic acid (DTNB) and 900 μl of 0.1 M potassium phosphate buffer

(pH 7.4). The yellow colored complex was formed due to the reaction of reduced glutathione with DTNB. At 412 nm absorbance was immediately read and the GSH activity was presented by μM GSH/g tissue.

3.2.15.2.4 Estimation of catalase

CAT activity determination was based on the process which depends on the decomposition of H_2O_2 . The reaction mixture was prepared by the addition of 100 μl of 5.9 mM H_2O_2 , 625 μl of 50 mM potassium phosphate buffer (pH 5.0) and 25 μl of tissue homogenate. Disappearance of H_2O_2 by catalase was measured in the reaction mixture at 240 nm spectrophotometrically. Absorbance change of 0.01 as units/min defines one unit CAT activity (Chance & Maehly, 1955)

3.2.16 Tissue Histology

Histological examination was also carried out on samples harvested from the test groups and control. This was done according to the method of used by Orieki et al., (2019). Briefly, slices of the tissues were fixed in 10% bouins solutions for 6 hours before changing to 10% formalin solution another 48 hours. The specimens were processed by placing them in ascending grades of alcohol in the following order, 50% alcohol for 1 hour, 70% alcohol for 1 hour, first 95% alcohol for 1 hour and second 95% alcohol for 1 hour 15 minutes, first absolute alcohol for one and half hours and second absolute alcohol for 2 hours to ensure proper dehydration of the tissues. The dehydrated tissues were then transferred to mixture of equal volumes of alcohol and xylene where they were left overnight and later cleared with two changes of xylene for 1 hour each. They were then infiltrated twice for 1 hour each with molten paraffin wax in the oven at 60°C . The tissues were then embedded in paraffin wax, trimmed and mounted on wooden chuck, and then taken to the microtome for sectioning at $5\mu\text{m}$ thickness. The sections were floated in floating-out bath from where they were picked with clean albuminized slides. The slides were placed in a staining dish and excess wax was removed by two changes of xylene,

hydrated by descending grades of alcohol in the order- absolute alcohol, 95% alcohol and 70% alcohol for 2 minutes each. The slides were taken to water and then stained by infiltrated Ehrlich haematoxylin for 15 minutes, and then washed in water for 5 minutes, differentiated in 10% acid alcohol and blued in running tap for 10 minutes. They were then counter stained with filtered eosine for 2 minutes. Excess eosine was removed in ascending grades of alcohol in the order- 75% alcohol, 95% alcohol and absolute alcohol for 2 minutes each. They were then cleared in two changes of xylene and each was cover slipped with depexmountant. The slides were viewed under a light microscope and selected images were captured using moticam 2.0 digital camera attached to a computer.

3.3. Statistical Analysis

The results were expressed as mean $\pm$ standard deviation. The statistical evaluation of data was performed using SPSS 7.5 for Windows. One way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparison were carried out on the data with value of $P < 0.05$ accepted as significant.

CHAPTER FOUR

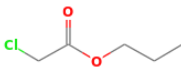
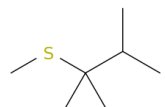
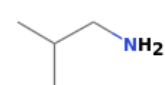
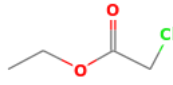
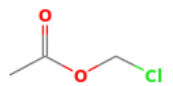
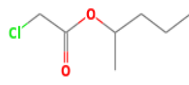
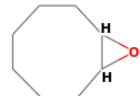
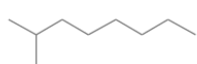
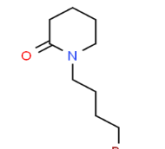
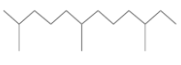
RESULTS AND DISCUSSION

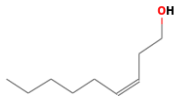
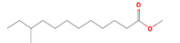
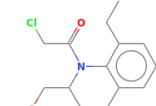
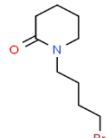
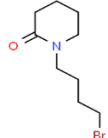
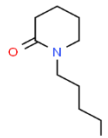
4.1 Results

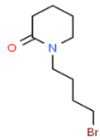
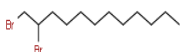
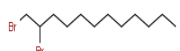
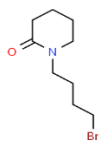
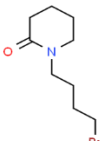
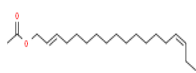
4.1.1 GC-MS Analysis of plant parts of *A.heterophyllus*

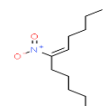
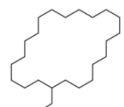
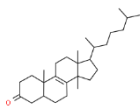
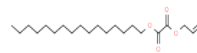
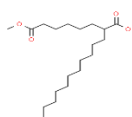
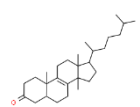
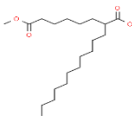
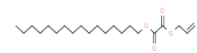
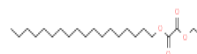
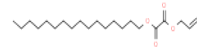
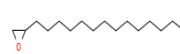
GC–MS profiling of ethanol stem bark extract of *A. heterophyllus* revealed sixty four (64) compounds (0.01–12.67% abundance, 6.659–36.914 min), chiefly long-chain fatty acid derivatives, alkenes, and sterol-related molecules. Key components were 13-docosenoic acid methyl ester, 1-docosene, and octanedioic acid derivatives. While many showed bioactivities like antimicrobial, antioxidant, insecticidal, or anti-inflammatory effects, others, mainly minor chloroacetate esters and hydrocarbons, were industrially relevant, highlighting both ecological and potential biomedical significance.

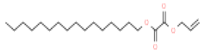
Table 4.1.: GC-MS characterisation of ethanol stem bark extract of *A. heterophyllus*

Peak	Retention Time(minutes)	Compound	% concentration	Structure
1	6.659	Chloroacetic acid, propyl ester	0.43	
2	8.104	2-Methylthio-2,3-dimethylbutane	0.22	
3	9.275	Isobutylamine	0.31	
4	12.374	Acetic acid, chloro-, ethyl ester	0.34	
5	15.374	Methanol, chloro-, acetate	0.43	
6	15.398	S-(Propoxythiocarbonyl)thiohydroxylamine	0.19	
7	17.006	Acetic acid, chloro-, 1-methylbutyl ester	0.22	
8	17.053	S-(Isopropoxythiocarbonyl)thiohydroxylamine	0.10	
9	19.383	9-Oxabicyclo[6.1.0]nonane, cis-	0.22	
10	19.585	Octane, 2-methyl-	0.01	
11	20.222	2-Piperidinone, N-[4-bromo-n-butyl]-	4.30	
12	21.997	Dodecane, 2,6,10-trimethyl-	0.12	
13	23.023	Cyclotetradecane	0.06	

14	23.819	3-Nonen-1-ol, (Z)-	2.34	
15	24.189	Tetradecane, 1-fluoro-	0.07	
16	26.460	Dodecanoic acid, 10-methyl-, methyl ester	0.22	
17	26.620	1-Heptadec-1-ynyl- cyclopentanol	0.19	
18	27.023	Metolachlor	2.43	
19	27.454	Metolachlor	0.34	
20	27.909	2-Piperidinone, N-[4-bromo-n- butyl]-	3.41	
21	28.760	Cyclopentane, (2-hexyloctyl)-	0.02	
22	28.825	Z-2-Octadecen- 1-ol	0.23	
23	29.151	E-8-Methyl-9- tetradecen-1-ol acetate	0.20	
24	29.243	Z-2-Octadecen- 1-ol	0.20	
25	29.351	Octadecanal	0.05	
26	29.401	2-Piperidinone, N-[4-bromo-n- butyl]-	0.23	
27	29.472	2-Piperidinone, N-[4-bromo-n- butyl]-	0.19	
28	29.571	Oxirane, tetradecyl-	0.45	

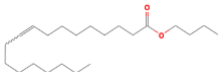
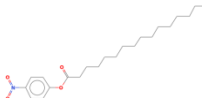
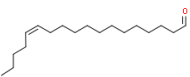
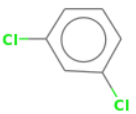
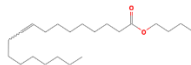
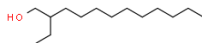
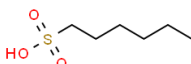
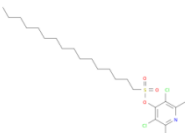
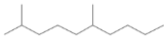
29	29.845	1-Docosene	0.34	
30	29.916	2-Piperidinone, N-[4-bromo-n-butyl]-	0.14	
31	30.609	Octadecanal	9.74	
32	31.920	Dodecane, 1,2-dibromo-	0.11	
33	31.018	Dodecane, 1,2-dibromo-	0.24	
34	31.155	2-Piperidinone, N-[4-bromo-n-butyl]-	0.31	
35	31.213	1-Docosene	0.38	
36	31.318	2-Piperidinone, N-[4-bromo-n-butyl]-	0.39	
37	31.376	E,Z-2,15-Octadecadien-1-ol acetate	0.34	
38	31.597	1-Docosene	1.34	
39	31.949	13-Docosenoic acid, methyl ester	12.67	
40	32.246	1-Docosene	1.54	
41	32.332	13-Docosenoic acid, methyl ester	0.61	
42	32.477	13-Docosenoic acid, methyl ester	1.74	
43	32.710	1-Docosene	12.63	
44	32.985	13-Docosenoic acid, methyl ester	2.43	
45	33.082	13-Docosenoic acid, methyl ester	1.05	
46	33.104	13-Docosenoic acid, methyl ester	0.32	

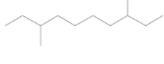
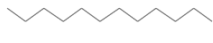
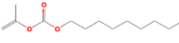
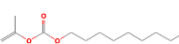
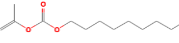
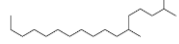
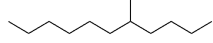
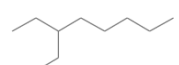
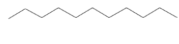
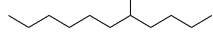
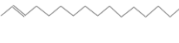
47	33.410	Cyclotetradecane, 1,7,11-trimethyl-4-(1-methylethyl)-	3.54	
48	33.590	1-Docosene	0.65	
49	33.661	6-Nitroundec-5-ene	0.65	
50	33.710	Cyclodocosane, ethyl-	0.49	
51	33.777	5.alpha.-Cholest-8-en-3-one, 14-methyl-	4.64	
52	33.821	Oxalic acid, allyl hexadecyl ester	2.30	
53	33.883	13-Docosenoic acid, methyl ester	0.44	
54	34.321	Octanedioic acid, 2-undecyl-, dimethyl ester	12.21	
55	34.554	13-Docosenoic acid, methyl ester	0.32	
56	34.682	5.alpha.-Cholest-8-en-3-one, 14-methyl-	2.62	
57	34.793	Octanedioic acid, 2-undecyl-, dimethyl ester	0.69	
58	34.860	Tetradecane, 1-chloro-	0.50	
59	35.038	Oxalic acid, allyl hexadecyl ester	1.25	
60	35.304	Cyclopentane, (4-octyldodecyl)-	8.22	
61	35.965	Oxalic acid, allyl octadecyl ester	0.18	
62	36.787	Oxalic acid, allyl hexadecyl ester	0.01	
63	36.858	Oxirane, tetradecyl-	3.00	

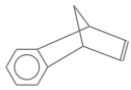
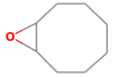
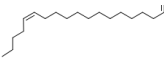
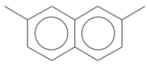
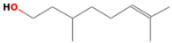
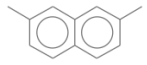
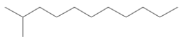
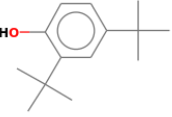
64	36.914	Oxalic acid, allyl hexadecyl ester	0.00	
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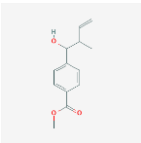
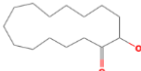
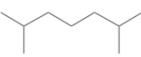
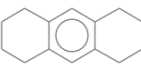
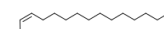
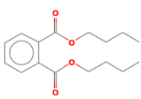
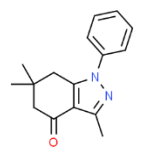
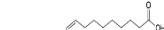
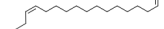
GC–MS profiling of ethanol pulp extract of *A. heterophyllus* revealed ninety four (94) distinct compounds ranging from hydrocarbons, esters, fatty acids, aldehydes, alcohols, phthalates, and sterols. The dataset is dominated by aliphatic hydrocarbons (decane, undecane, dodecane derivatives), long-chain fatty acid esters (hexadecanoic acid, methyl ester; butyl 9-octadecenoate), and unsaturated fatty acids (oleic acid, cis-vaccenic acid, linoleic acid derivatives).

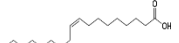
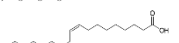
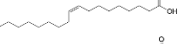
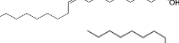
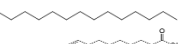
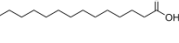
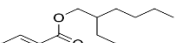
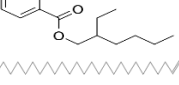
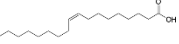
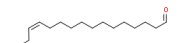
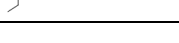
Table 4.2. GC-MS characterisation of ethanol pulp extract of *A. heterophyllus*

Peak	Retention Time(minutes)	Compound	% concentration	Structure
1	5.250	Butyl 9-octadecenoate	1.22	
2	5.455	2-Thiopheneacetic acid, 3-tridecyl ester	0.13	
3	6.885	9-Octadecenal	1.43	
4	7.137	Hexadecanoic acid, 4-nitrophenyl ester	0.10	
5	7.225	13-Octadecenal	0.43	
6	7.425	Benzene 1,3-dichloro-	1.31	
7	7.788	Octadecane, 1-(ethenyloxy)-	0.34	
8	8.362	Butyl 9-octadecenoate	0.60	
9	8.521	2-Ethyl-1-dodecanol	1.23	
10	8.612	1-Hexadecanesulfonic acid	2.34	
11	8.876	Hexadecane, 3-methyl- 3,5--dichloro-2-6-dimethyl-4-pyridyl ester	3.38	
12	8.980	Nonane	1.84	
13	9.059	Decane	1.48	
14	9.138	2,6-Dimethyldecane	1.69	
15	9.217	Tricosane, 2-methyl-	0.96	

16	9.430	Decane, 3,8-dimethyl	7.72	
17	9.558	Decane, 3,8-dimethyl	1.56	
18	9.622	Undecane	2.78	
19	9.737	Dodecane	3.23	
20	9.816	Heptadecane, 2,6,10,14-tetramethyl	4.54	
21	9.938	Carbonic acid, nonyl prop-1-en-2-yl ester	1.18	
22	9.938	Carbonic acid nonyl prop-1-en-2-yl ester	1.24	
23	10.159	Carbonic acid nonyl prop-1-en-2-yl ester	3.34	
24	10.268	Heptadecane, 2,6-dimethyl-	4.35	
25	10.506	Undecane, 5-methyl-	1.70	
26	10.579	Tricosane, 2-methyl-	1.27	
27	10.635	Octane, 3-ethyl-	1.52	
28	10.992	Stearic acid hydrazide	0.21	
29	11.119	Undecane	0.23	
30	11.336	Undecane, 5-methyl-	0.35	
31	11.468	Oxirane, tetradecyl-	0.15	
32	11.563	1-Octadecanesulphonyl chlorid	0.18	
33	11.710	Stearic acid hydrazide	0.07	
34	12.584	E-14-Hexadecenal	0.95	
35	12.803	Dodecane	1.34	
36	13.191	Trifluoroacetoxy hexadecane	0.24	
37	14.651	Stearic acid hydrazide	0.17	

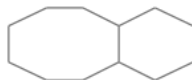
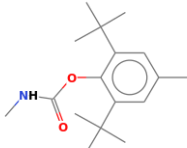
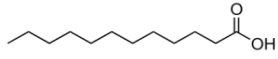
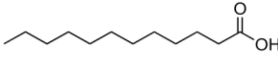
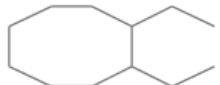
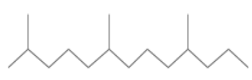
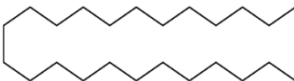
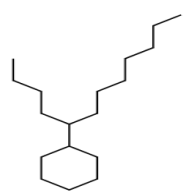
38	14.888	Decyl octyl ether	0.57	
39	15.706	Tridecane	1.82	
40	15.873	1,4-Methanonaphthalene, 1,4-dihydro-	0.84	
41	16.361	9-Oxabicyclo[6.1.0]nonane	0.34	
42	17.108	9-Octadecenal, (Z)-	0.17	
43	17.473	Hexadecane, 1-chloro-	0.26	
44	17.662	13-Octadecenal, (Z)-	0.27	
45	17.804	Oxirane, decyl-	0.23	
46	18.283	4-Tetradecene, (Z)-	1.58	
47	18.476	Tridecane	1.33	
48	18.963	Naphthalene, 2,7-dimethyl-	0.10	
49	19.354	Citronellol	0.23	
50	19.437	Naphthalene, 2,7-dimethyl-	0.57	
51	19.743	1-Hexacosanol	0.15	
52	20.087	9-Octadecenal	0.42	
53	20.899	Dodecane, 1,1'-oxybis-	0.13	
54	21.104	Undecane, 2-methyl-	0.88	
55	22.130	2,4-Di-tert-butylphenol	8.18	
56	23.440	Z-8-Hexadecene	2.35	
57	23.597	Hexadecane	0.57	

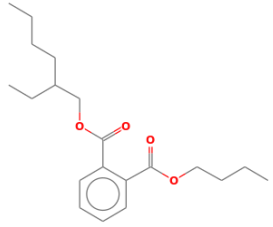
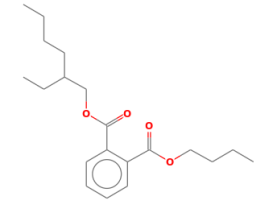
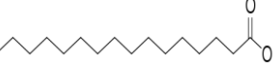
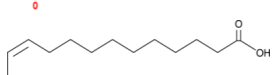
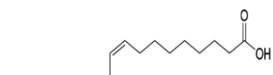
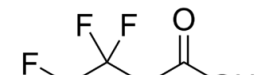
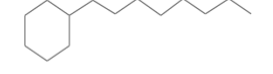
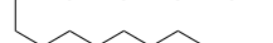
58	23.743	Cetene	0.31	
59	23.978	Cyclohexadecane	0.37	
60	25.513	Benzoic acid, 4-(3-hydroxy-3-methyl-1-butynyl)-, methyl ester	0.32	
61	25.958	Cyclopentadecanone, 2-hydroxy-	0.17	
62	26.072	Heptane, 2,6-dimethyl-	0.41	
63	28.090	1-Octadecene	2.66	
64	28.325	1-Octadecene	0.35	
65	28.553	3-Eicosene, (E)-	0.28	
66	29.799	Anthracene, 1,2,3,4,5,6,7,8-octahydro-1,1,4,4,5,5,8,8-Octamethyl-	0.47	
67	29.928	cis-Vaccenic acid	0.18	
68	30.069	Hexadecanoic acid, methyl ester	1.02	
69	30.415	13-Octadecenal, (Z)-	0.23	
70	30.527	Dibutyl phthalate	1.31	
71	30.703	1-Octadecene	2.05	
72	30.886	Indazol-4-one, 3,6,6-trimethyl-1-phthalazin-1-yl-1,5,6,7-tetrahydro-	0.60	
73	31.033	Oleic Acid	0.22	
74	31.185	13-Octadecenal, (Z)-	0.10	
75	31.551	9,12-Octadecadienoic acid, methyl ester	1.43	
76	31.762	cis-Vaccenic acid	0.30	

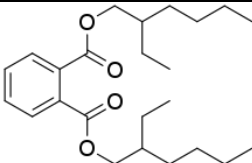
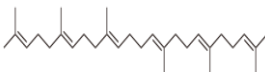
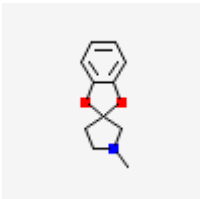
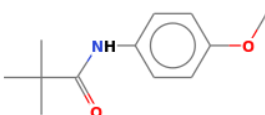
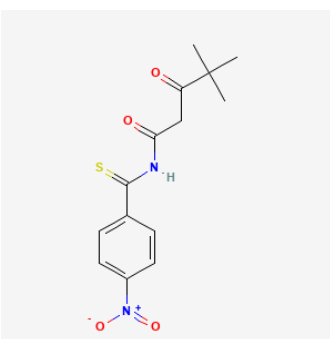
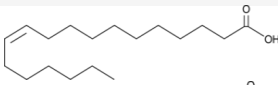
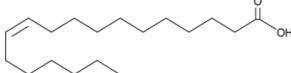
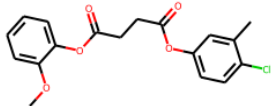
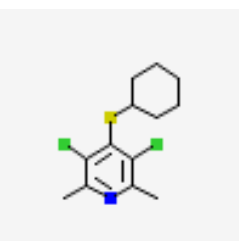
77	31.849	Oleic acid	0.05	
78	32.035	Oleic acid	0.51	
79	32.181	1-Docosene	1.03	
80	32.340	Oleic Acid	0.09	
81	32.605	Oleic Acid	0.53	
82	32.843	Oleic Acid	0.61	
83	32.929	Oleic Acid	0.31	
84	33.103	Oleic Acid	0.53	
85	33.229	Oleic Acid	0.40	
86	33.375	9-Tricosene, (Z)-	1.08	
87	33.550	Hexacosanoic acid	0.20	
88	33.678	Oleic Acid	0.24	
89	34.001	Oleic Acid	0.25	
90	34.116	Tetradecanoic acid, dodecyl ester	0.36	
91	34.543	Bis(2-ethylhexyl) phthalate	1.50	
92	34.796	1-Hexacosene	0.41	
93	36.839	Oleic acid	0.20	
94	37.248	Octadecenal, (z)-	0.33	

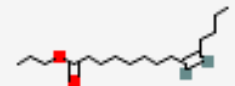
The GC–MS profiling of ethanol leaf extract of *A. heterophyllus* analysis revealed forty eight (48) compounds with diverse chemical classes, including fatty acids (e.g., palmitic, oleic, vaccenic acids), alkenes (eicosene, docosene), hydrocarbons, esters, and sterol-related molecules (squalene).

Table 4.3. GC-MS characterisation of ethanol leaf extract of *A. heterophyllus*

PK	RT (minutes)	Compound	Area Pct	Structure
1	6.8623	Cyclooctane, 1,2-diethyl-	0.54	
2	7.0011	Tetradecane	0.89	
3	8.4896	Phenol, 2,6-bis(1,1-dimethylethyl)-	0.65	
4	8.6109	Hexadecane	0.51	
5	9.5595	Dodecanoic acid	1.78	
6	9.7361	Dodecanoic acid	0.23	
7	10.0255	1-Tridecene	0.65	
8	10.1266	Cyclooctane, 1,2-diethyl-	0.34	
9	10.1893	Hexadecane	1.93	
10	10.8875	1-Hexacosanol	0.23	
11	10.9921	Tetradecane, 1-fluoro-	0.65	
12	11.7127	Heptadecane	0.86	
13	11.8457	2,6,10-Trimethyltridecane	0.84	
14	11.919	Tetracosane	0.38	
15	12.5291	Tetradecanoic acid	2.54	
16	13.0218	9-Eicosene, (E)-	1.21	
17	13.1685	Hexadecane	1.65	
18	13.3326	Hexadecane, 1-chloro-	0.65	
19	13.9125	Dodecane, 5-cyclohexyl-	0.28	

20	14.5649	Nonadecane	0.23	
21	14.7123	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	0.78	
22	15.316	n-Hexadecanoic acid	4.65	
23	15.7725	5-Eicosene, (E)-	1.27	
24	15.8975	Nonadecane	0.36	
25	17.4737	9-Octadecenoic acid	5.34	
26	17.6435	2-Chloropropionic acid, hexadecyl ester	1.17	
27	17.8409	cis-Vaccenic acid	0.51	
28	17.8732	Oleic Acid	0.44	
29	17.9526	Heptadecyl heptafluorobutyrate	0.54	
30	18.2928	1-Docosene	2.29	
31	18.4016	Tritetracontane	0.58	
32	18.5206	9-Eicosene, (E)-	0.21	
33	19.0821	Octadecane, 1-(ethenyloxy)-	0.16	
34	19.1959	Decane, 3-cyclohexyl-	0.42	
35	19.5793	Carbonic acid, eicosyl vinyl ester	0.22	
36	20.6082	1-Octadecene	0.98	
37	20.707	Tritetracontane	0.20	

38	21.7858	Bis(2-ethylhexyl) phthalate	0.70	
39	24.8635	Squalene	1.08	
40	31.3241	Pyrrolidine, 1-methyl- 3,2'-spiro-benzo-1,3- dioxolane-	7.46	
41	32.097	Propanamide, N-(3- methoxyphenyl)-2,2- dimethyl-	0.69	
42	32.2001	pentanamide, 4,4- dimethyl-N-(4- nitrophenyl)-3-oxo-	1.02	
43	32.3524	cis-Vaccenic acid	2.62	
44	32.556	cis-Vaccenic acid	2.69	
45	32.9484	Succinic acid, 4- chloro-3- methylphenyl 2- methoxyphenyl ester	19.02	
46	32.9781	2,6-Lutidine, 3,5- dichloro-4- cyclohexylthio-	11.52	

47	36.6347	cis-9-Tetradecenoic acid, propyl ester	1.17	
48	37.2479	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	16.85	

4.1.2 Fourier Transform Infrared Spectroscopy (FTIR) analysis results

FTIR analysis was used for identifying surface functional groups of the stem bark of *A.heterophyllus*.

Table 4.4. represents the FT-IR interpretation of the stem bark extract of *A.heterophyllus*. The presence of absorptions in the 3600 to 3100 cm⁻¹ region indicates that the compound contains O-H or N-H groups. The bands in the 3100 to 3000 cm⁻¹ region indicate the presence of hydrogens bonded to sp²-hybridized carbons, so the compound must have one or more CC double bonds. The presence of absorptions in the 3000 to 2850 cm⁻¹ region indicate that there are hydrogens bonded to sp³-hybridized carbons in the compound. Examination of the triple-bond region shows indications of the absence of triple bonded functional group. Continuing to the double-bond region, the strong absorption at 1618 cm⁻¹ indicates the presence of a carbonyl group. This is not part of a carboxylic acid (no O-H) or an aldehyde (absence of absorptions in the 2830–2700 cm⁻¹ region). Nor does the unknown appear to be an amide (no N-H, carbonyl absorption too high), or an acyl chloride (carbonyl position too low). This indicates ketone possibility. The strong absorption at 1176 cm⁻¹ suggests that the unknown is an amine.

Table 4.4. The FT-IR interpretation of the stem bark extract of *A.heterophyllus*

Wave number	Peak intensity	Peak Shape	Bond	Compound
698.7069	Medium	Sharp	Sp ² C- H bending	Monosubstituted Benzene
826.8491	Strong	Broad	Sp ² C-H bending	Trisubstituted alkene
1019.69	Medium	Sharp	C-F stretching	Fluro compounds
1176.327	Medium	Sharp	C-N stretching	Amine
1384.815	Strong	Broad	O-H stretching	Alcohol
1618.791	Medium	Very sharp	C=C, C=O stretching	Alkene, β Lactam
2086.489	Strong	Narrow	N=C=S stretching	Isothiocyanate
2273.714	Strong	Sharp	N=C=O stretching	Isocyanate
2446.047	Strong	Very sharp	N-H stretching	Ammonium
2678.061	medium	Broad	C-H stretching	Aldehyde
3039.566	Medium	Sharp	C-H stretching	Alkene
3162.679	Medium	Sharp	O-H stretching	Carboxylic acid
3281.142	Weak	Broad	O-H stretching	Alcohol
3429.525	Medium	Sharp	N-H stretching	Primary amine
3822.704	Medium	Sharp	O-H stretching	Free alcohol

FTIR characterization of ethanol leaf extract of *A.heterophyllus* showed single bond area (2500-4000 cm⁻¹), several gaps were detected as shown in **table 4.5**. Broad absorption band in the range of between 3650 and 3250 cm⁻¹, indicating hydrogen bond. Peaks at between 3000 and 3200 cm⁻¹, replying the aromatic ring. Peaks at below 3000 cm⁻¹, responding the single bond of carbon. The absorptions in the region of 3000 to 2850 cm⁻¹ indicate the presence of H's bonded to sp³-hybridized C's. Aldehyde peak was detected at between 2700 and 2800 cm⁻¹. Regarding the triple bond region (2000-2500 cm⁻¹), no peak was detected, informing no C≡C bonding.

In the double bond region (1500-2000 cm⁻¹), several peaks were detected: The weak absorption at 1662 cm⁻¹ indicates the presence of a aromatic compound. This is not part of a carboxylic acid (no O-H) or an aldehyde (absence of absorptions in the 2830–2700 cm⁻¹ region). Nor does the unknown appear to be an amide (no N-H, carbonyl absorption too high), an anhydride (absence of a second carbonyl band), or an acyl chloride (carbonyl position too low). This leaves a ketone or an ester as possibilities. In the fingerprint region (600-1500 cm⁻¹), strong signal was found at about 900 cm⁻¹ indicating the presence of alkene

Table 4.5. FTIR characterization of ethanol leaf extract of *A.heterophyllus*

Wave number	Peak intensity	Peak shape	Bond	Compound
900.7966	Strong	Broad	C=C bending	Alkene
1047.5	Strong	Sharp	C-O stretching	Primary alcohol
1369.536	Strong	Sharp	S=O stretching	Sulfonate
1473.258	Weak	Broad	N-O stretching	Nitro compound
1664.323	Weak	Broad	C-H stretching	Aromatic compound
2020.284	Strong	Broad	N=C=S stretching	Isothiocyanate
2353.372	Strong	Sharp	O=C=O stretching	Carbon dioxide
2859.711	Strong	Sharp	C-H stretching	Alkane
3002.457	Strong	Sharp	C-H stretching	Alkene
3289.143	Medium	Broad	O-H stretching	Carboxylic acid
3772.403	Strong	Broad	O-H stretching	Free alcohol

The FTIR analysis of pulp extract is shown in the **table 4.6** below. The result showed that the sample contain several functional groups with characteristic band observed around 3900 cm^{-1} - 3200 cm^{-1} indicating the presence of hydrogen bonding in the sample. This the presence of O-H group at this region is due to the vibration of water molecules. Peaks at between 3000 and 3200 cm^{-1} , replying the aromatic ring. Peaks at below 3000 cm^{-1} , responding the single bond of carbon. The absorptions in the region of 3000 to 2850 cm^{-1} attributed to presence of aliphatic C-H stretch of CH, CH₂ and CH₃ groups. The peak observed at 2084 cm^{-1} arises due to the presence of C≡C groups. The peak at 1632 cm^{-1} corresponded to C = C stretching of alkene. The strong absorption at 1395 cm^{-1} indicates the presence of alkane. The presence of aromatic compound was confirmed due to the presence of peak at 1277 cm^{-1} and 749 cm^{-1} .

Table 4.6. FTIR characterisation of ethanol pulp extract of *A. heterophyllus*

Peak	Peak Intensity	Peak Shape	Bond	Compound
749.1453	Strong	Broad	C-H out of plane bending	1,2 disubstituted aromatic compound
1277.746	Medium	Sharp	C-N or C-O stretching	Aromatic amine or aromatic ester
1395.621	Strong	Sharp	C-H bending	Alkane
1632.987	Strong	Sharp	C=C stretching	Alkene
2084.933	Strong	Broad	C≡C stretching	Alkynes
2625.22	Weak	Broad	O-H stretching	Alcohol
2778.068	Medium	Sharp	C-H stretching	Aldehyde
2912.32	Strong	Sharp	C-H stretching	Alkane
32011.296	Weak	Sharp	O-H stretching	Alcohol
3391.093	Medium	Sharp	N-H stretching	Aliphatic primary amine
3802.793	Medium	Sharp	O-H stretching	Free alcohol

4.1.3 Amino acid evaluation of ethanol extract of stem bark, leaf and pulp extract of *A. heterophyllus*

Table 4.7. revealed the amino acid composition in mean $\pm$ standard deviation. Values with different superscript letters within a row are significantly different ($p < 0.05$). "-" indicated not detected (ND) in that sample. The statistical differences ($p < 0.05$) confirmed that each plant part had a distinct amino acid profile. While bark provided a reservoir of structural and metabolic amino acids, the leaves contributed more nutritionally essential amino acids, and the pulp offered specialized amino acids with potential health benefits. Together, this indicated that different parts of the plant complemented each other nutritionally and could serve diverse roles in food and therapeutic applications

Table 4.7 Amino acid profile of ethanol extract of stem bark, leaf, and pulp extract of *A. heterophyllus*

Amino acids	Concentration ppm		
	Stem Bark	Leaf	Pulp
Proline	42.16 $\pm$ 0.02 ^a	1.87 $\pm$ 0.02 ^c	2.96 $\pm$ 0.00 ^b
Serine	15.88 $\pm$ 0.01 ^a	8.60 $\pm$ 0.02 ^b	0.98 $\pm$ 0.01 ^c
Glycine	14.90 $\pm$ 0.02 ^a	-	0.74 $\pm$ 0.01 ^b
Alanine	12.15 $\pm$ 0.00 ^a	7.74 $\pm$ 0.02 ^b	0.57 $\pm$ 0.00 ^c
Threonine	11.74 $\pm$ 0.00 ^b	16.39 $\pm$ 0.01 ^a	5.25 $\pm$ 0.02 ^c
Leucine	4.11 $\pm$ 0.01 ^a	1.61 $\pm$ 0.02 ^c	1.73 $\pm$ 0.02 ^{bc}
Isoleucine	3.90 $\pm$ 0.01 ^b	10.06 $\pm$ 0.02 ^a	3.92 $\pm$ 0.01 ^b
Valine	2.82 $\pm$ 0.02 ^b	5.33 $\pm$ 0.05 ^a	2.82 $\pm$ 0.02 ^b
Argenine	0.81 $\pm$ 0.01 ^c	9.16 $\pm$ 0.02 ^a	8.44 $\pm$ 0.02 ^b
Lysine	0.64 $\pm$ 0.01 ^c	3.41 $\pm$ 0.01 ^a	0.93 $\pm$ 0.02 ^b
Methionine	0.61 $\pm$ 0.00 ^b	0.92 $\pm$ 0.02 ^a	0.73 $\pm$ 0.02 ^b
Tryptophan	0.42 $\pm$ 0.02 ^c	1.76 $\pm$ 0.01 ^b	3.61 $\pm$ 0.01 ^a
Glutamate	0.29 $\pm$ 0.01 ^c	2.75 $\pm$ 0.00 ^a	0.75 $\pm$ 0.02 ^b
Aspartate	0.30 $\pm$ 0.01 ^c	0.96 $\pm$ 0.02 ^a	0.33 $\pm$ 0.02 ^{bc}
Cystine	0.27 $\pm$ 0.01 ^c	1.32 $\pm$ 0.02 ^a	0.32 $\pm$ 0.03 ^b
Tyrosine	-	3.80 $\pm$ 0.02 ^b	29.70 $\pm$ 0.02 ^a
Phenylalanine	-	1.52 $\pm$ 0.00 ^b	3.32 $\pm$ 0.02 ^a
Histidine	-	1.16 $\pm$ 0.02 ^a	1.12 $\pm$ 0.01 ^a

Values are mean $\pm$ standard deviation. Values with different superscript letters within a row are significantly different ($p < 0.05$). "-" indicates not detected (ND) in that sample

4.1.4 Fatty Acid Analysis of ethanol extract of stem bark, leaf, and pulp extract of *A. heterophyllus*.

The result showed clear statistical variations ($p < 0.05$) in fatty acid composition across stem bark, leaf, and pulp. Stem bark was dominated by oleic acid ($57.15 \pm 0.01\%$), which was significantly higher than in leaf and pulp. Leaf contained significantly higher levels of elaidic acid ($17.99 \pm 0.01\%$), trans-oleic acid ($15.82 \pm 0.02\%$), and lauric acid ($8.74 \pm 0.02\%$), compared to the other parts. Pulp showed the highest concentrations of stearic acid ($17.11 \pm 0.01\%$), trans-oleic acid ($17.09 \pm 0.00\%$), and arachidonic acid ($4.37 \pm 0.03\%$), all significantly different from stem bark and leaf. Some fatty acids such as EPA and DHA were present in lower but statistically distinct amounts across all samples

Table 4.8 Fatty acid profile of ethanol stem bark, leaf, and pulp extract of *A. heterophyllus*

Fatty acids	Fatty acid names	% Composition		
		Stem Bark	Leaf	Pulp
C18:1c	Oleic acid (cis)	57.15 ± 0.01 ^a	20.23 ± 0.01 ^c	21.77 ± 0.01 ^b
C18:2c	Linoleic acid (cis)	6.67 ± 0.04 ^b	7.81 ± 0.04 ^a	3.72 ± 0.04 ^c
C18:3	α -Linolenic acid	5.70 ± 0.04 ^a	4.77 ± 0.00 ^b	2.83 ± 0.00 ^c
C14	Myristic acid	4.80 ± 0.02 ^c	7.46 ± 0.01 ^b	9.03 ± 0.04 ^a
C18:0	Stearic acid	4.64 ± 0.03 ^c	5.18 ± 0.03 ^b	17.11 ± 0.01 ^a
C18:2t	Elaidic acid (trans)	4.08 ± 0.01 ^c	17.99 ± 0.01 ^a	5.55 ± 0.00 ^b
C18:1t	Trans-oleic acid	3.84 ± 0.04 ^c	15.82 ± 0.02 ^b	17.09 ± 0.00 ^a
C20	Arachidic acid	3.38 ± 0.00 ^a	2.01 ± 0.01 ^c	2.19 ± 0.02 ^b
C12	Lauric acid	2.98 ± 0.02 ^c	8.74 ± 0.01 ^a	2.70 ± 0.03 ^b
C20:5	Eicosapentaenoic acid (EPA)	2.37 ± 0.01 ^b	1.82 ± 0.01 ^c	2.60 ± 0.01 ^a
C20:3	Dihomo- γ -linolenic acid	1.50 ± 0.03 ^b	1.63 ± 0.02 ^a	0.57 ± 0.03 ^c
C16	Palmitic acid	1.14 ± 0.01 ^c	0.43 ± 0.001 ^b	7.34 ± 0.03 ^a
C22:6	Docosahexaenoic acid (DHA)	0.67 ± 0.02 ^c	1.28 ± 0.02 ^a	0.63 ± 0.00 ^b
C22	Behenic acid	0.62 ± 0.01 ^c	2.90 ± 0.00 ^a	2.51 ± 0.01 ^b
C20:4	Arachidonic acid	0.40 ± 0.01 ^c	1.94 ± 0.04 ^b	4.37 ± 0.03 ^a

The fatty acid composition varies distinctly across the stem bark, leaf, and pulp. The result is presented as mean ± standard deviation. Different superscript letters (□, □, □) in the same row indicate significant differences ($p < 0.05$) across plant parts, while same letters mean no difference.

4.1.5 Gas Chromatography - Flame Ionization Detector (GC-FID) characterisation of ethanol extract of stem bark, leaf and pulp extract of *A. heterophyllus*

The GC-FID analysis of the stem bark extract revealed that α -Naphthol (10.02 ppm) and 3,5-Hydroxy toluene (7.61 ppm) were the most abundant phenolic compounds, followed by Picric acid (4.97 ppm) and Flavonones (4.72 ppm). Most compounds were present in moderate to low concentrations, such as Resveratrol (2.99 ppm) and Quinol (3.10 ppm), while some occurred only in trace amounts like Assculetin (0.29 ppm) and Phenetol (0.47 ppm).

Table 4.9 GC-FID characterisation of the ethanol stem bark extract of *A. heterophyllum*

S/No	Ret Time (min)	Amount (ppm)	Name
1	3.266	0.672	Catechol
2	4.177	1.467	Resorcinol
3	5.022	2.046	Hydroxyquinol
4	5.513	0.891	Pyrogallol
5	5.945	3.102	Quinol
6	6.165	1.696	Phloroglucinol
7	6.328	2.671	p-Cresol
8	6.644	7.614	3,5-Hydroxy toluene
9	6.917	0.465	Phenetol
10	7.258	10.019	alpha-Naphthol
11	7.628	0.821	Anisole
12	7.823	4.966	Picric acid
13	8.198	2.088	2-Hydroxy phenol
14	8.466	4.724	Flavonones
15	8.691	2.005	Xanthone
16	8.901	0.537	(+)-Pinoresinol
17	9.305	1.891	Lignans
18	10.353	0.285	Assculetin
19	11.526	0.846	Cinnamic acid
20	12.674	2.990	Resveratrol

The GC-FID profile of the ethanol leaf extract of *A.heterophyllus* shows that p-Cresol (71.95 ppm), Phloroglucinol (66.20 ppm), and Hydroxyquinol (53.51 ppm) were the dominant compounds, followed by Pyrogallol (49.22 ppm) and Quinol (29.87 ppm). These were mainly strong antioxidants with additional antimicrobial, cytotoxic, and anticancer roles, suggesting they drive the extract's bioactivity. Other compounds such as Resorcinol, Lignans, and Phenetol occur in smaller amounts but still contribute important pharmacological properties.

Table 4.10 GC-FID characterisation of the ethanol leaf extract of *A. heterophyllus*

S/No	Ret Time (min)	Amount (ppm)	Name
1	4.127	4.542	Resorcinol
2	5.093	53.510	Hydroxyquinol
3	5.524	49.220	Pyrogallol
4	5.916	29.865	Quinol
5	6.110	66.203	Phloroglucinol
6	6.278	71.948	p-cresol
7	6.654	8.722	3,5-Hydroxy toluene
8	6.922	7.380	Phenetol
9	7.207	3.028	alpha-Naphthol
10	8.247	1.624	2-Hydroxy phenol
11	9.271	3.449	Lignans

GC-FID result of the ethanol pulp extract revealed phenolic compounds with diverse bioactivities. Pyrogallol (32.81 ppm), Hydroxyquinol (24.23 ppm), and Quinol (21.17 ppm) were the most abundant, mainly contributing antioxidant and antimicrobial effects. Compounds like Phloroglucinol and Phenetol appeared in moderate amounts, while Resveratrol, Lignans, and α -Naphthol, though less abundant, are pharmacologically significant.

Table 4.11 GC-FID characterisation of the ethanol pulp extract of *A. heterophyllus*

S/No	Ret Time (min)	Amount (ppm)	Name
1	4.262	8.861	Resorcinol
2	4.866	21.165	Quinol
3	5.834	32.81388	Pyrogallol
4	6.148	24.22826	Hydroxyquin ol
5	6.585	12.963	Phloroglucin ol
6	6.843	2.363	p-cresol
7	7.142	6.814	3,5-Hydroxy toluene
8	7.424	10.548	Phenetol
9	7.800	4.801e-1	alpha- Naphthol
10	8.028	1.699	Anisole
11	8.769	2.005	Picric acid
12	9.037	1.123	2-Hydroxy phenol
13	9.545	1.599	Flavonones
14	11.062	1.342	Lignans
15	11.644	1.445	Assculetin
16	13.016	2.673e-1	Resveratro

4.1.6 Proximate analysis of the of ethanol extract of stem bark, leaf and pulp of *A. heterophyllus*

The proximate composition (**table 4.12**) showed the stem bark was richest in available carbohydrate ($62.07 \pm 0.04\%$) and ash ($10.47 \pm 0.02\%$), indicating high energy and mineral content. The leaf showed higher fibre ($8.99 \pm 0.01\%$) and balanced protein ($8.84 \pm 0.03\%$), while the pulp contained the highest moisture ($26.77 \pm 0.01\%$), fat & oil ($13.44 \pm 0.01\%$), and protein ($13.30 \pm 0.00\%$), making it more nutritious and energy-dense but less stable for storage due to high water content.

Table 4.12 Proximate composition of ethanol extract of the stem bark, leaf, and pulp of *A. heterophyllus*

Parameter	Concentration (%)		
	Stem Bark	Leaf	Pulp
Moisture	7.78 $\pm$ 0.01 ^c	12.53 $\pm$ 0.02 ^b	26.77 $\pm$ 0.01 ^a
Ash	10.47 $\pm$ 0.02 ^a	10.09 $\pm$ 0.03 ^b	3.01 $\pm$ 0.01 ^c
Fat & Oil	4.42 $\pm$ 0.00 ^c	10.42 $\pm$ 0.01 ^b	13.44 $\pm$ 0.01 ^a
Fibre	5.24 $\pm$ 0.02 ^b	8.99 $\pm$ 0.01 ^a	1.65 $\pm$ 0.04 ^c
Protein	10.02 $\pm$ 0.03 ^b	8.84 $\pm$ 0.03 ^c	13.30 $\pm$ 0.00 ^a
Avalable	62.07 $\pm$ 0.04 ^a	49.13 $\pm$ 0.02 ^b	41.87 $\pm$ 0.01 ^c
Carbohydrate			

Values are mean $\pm$ standard deviation. Means in the same row with different superscript letters (a–c) differ significantly ($p < 0.05$).

4.1.7 Vitamin analysis of ethanol extracts of stem bark, leaf and pulp of *A. heterophyllus*

The result showed that the leaves were the richest in vitamin D, while the stem bark was slightly higher in vitamin E compared to the leaves. The pulp was unique for its vitamin B12 content and higher vitamin C, but generally lower in fat-soluble vitamins (A, D, E)

Table 4.13 Vitamin composition of ethanol extract of the stem bark, leaf, and pulp of *A. heterophyllus*

Vitamins	Concentration (mg/100g)		
	Stem Bark	Leaves	Pulp
A	1.89 ± 0.02 ^a	1.95 ± 0.00 ^a	0.55 ± 0.01 ^b
B1	0.06 ± 0.00 ^a	0.06 ± 0.04 ^a	0.01 ± 0.00 ^b
B2	0.06 ± 0.00 ^a	0.06 ± 0.03 ^a	0.02 ± 0.00 ^b
B3	1.49 ± 0.01 ^a	1.20 ± 0.02 ^b	1.03 ± 0.03 ^c
B6	1.93 ± 0.04 ^c	2.22 ± 0.02 ^b	2.43 ± 0.01 ^a
B12	-	-	4.18 ± 0.01
C	4.39 ± 0.02 ^c	4.56 ± 0.01 ^b	6.01 ± 0.02 ^a
D	6.36 ± 0.01 ^b	9.33 ± 0.02 ^a	0.33 ± 0.01 ^c
E	97.91 ± 0.02 ^a	97.03 ± 0.04 ^a	1.43 ± 0.01 ^b

The vitamin analysis result is displayed in mean ± standard deviation. Different superscripts across rows indicate statistical significance ($p < 0.05$). B12 appears only in pulp, so no comparison is possible.

4.1.8 Mineral analysis of ethanol extract of stem bark, leaf and pulp of *A. heterophyllus*

There were significant ($p < 0.05$) variations in mineral composition among stem bark, leaf, and pulp (**Table 4.14**). Leaves consistently had higher levels of macronutrients (Mg, Ca, K, Na), denoted by different superscript letters from bark and pulp. Pulp showed significantly higher copper and molybdenum, while bark was distinct in its higher zinc, iron, and manganese. Minerals such as cobalt and silver showed no statistical difference (same letters). This indicated selective mineral accumulation across plant parts. The leaves generally had higher levels of essential minerals such as magnesium, calcium, potassium, and sodium compared to the stem bark and pulp. The pulp, however, contained the highest copper concentration and notable molybdenum. Toxic heavy metals (lead, cadmium, arsenic, mercury) were present in small amounts across all parts, with stem bark and leaves showing slightly higher values than the pulp. Overall, leaves were richer in essential minerals, while pulp provided unique contributions like copper and molybdenum.

Table 4.14 Mineral composition of ethanol stem bark, leaf, and pulp extract of *Artocarpus heterophyllus*

Minerals	Concentration (ppm)		
	Stem Bark	Leaf	Pulp
Chromium	0.12 ± 0.04 ^b	0.20 ± 0.01 ^a	0.04 ± 0.01 ^c
Arsenic	0.10 ± 0.01 ^a	0.05 ± 0.01 ^b	0.07 ± 0.01 ^b
Silver	0.02 ± 0.01 ^a	0.02 ± 0.04 ^a	-
Mercury	0.01 ± 0.02 ^b	0.02 ± 0.03 ^b	0.03 ± 0.03 ^a
Lead	0.18 ± 0.04 ^a	0.19 ± 0.01 ^a	0.09 ± 0.02 ^b
Cobalt	0.02 ± 0.02 ^a	0.02 ± 0.03 ^a	0.02 ± 0.03 ^a
Magnesium	8.18 ± 0.01 ^c	9.48 ± 0.02 ^a	8.79 ± 0.02 ^b
Calcium	8.21 ± 0.01 ^b	9.32 ± 0.02 ^a	-
Potassium	8.21 ± 0.01 ^b	9.04 ± 0.01 ^a	8.28 ± 0.01 ^b
Selenium	0.72 ± 0.02 ^a	0.61 ± 0.01 ^b	0.09 ± 0.01 ^c
Molybdenum	0.11 ± 0.02 ^b	0.09 ± 0.04 ^b	0.28 ± 0.03 ^a
Copper	0.05 ± 0.01 ^b	0.004 ± 0.02 ^c	0.37 ± 0.01 ^a
Zinc	0.96 ± 0.01 ^a	0.46 ± 0.02 ^b	ND
Cadmium	0.19 ± 0.03 ^a	0.10 ± 0.03 ^b	ND
Sodium	3.66 ± 0.02 ^c	7.18 ± 0.02 ^a	6.48 ± 0.05 ^b
Manganese	1.53 ± 0.03 ^a	0.93 ± 0.04 ^b	0.84 ± 0.04 ^b
Iron	0.46 ± 0.02 ^a	0.23 ± 0.06 ^b	ND
Nickel	1.02 ± 0.04 ^a	-	0.012 ± 0.02 ^b

The result of the mineral analysis is displayed in Table 4.8.1. The result is displayed in mean ± standard deviation. Different superscripts across rows indicate statistical significance.

4.1.9 *In vitro* antioxidant determination of ethanol extract of stem bark, leaf and pulp extract of *A. heterophyllus*

4.1.9.1 Nitric oxide concentration of plant parts

Nitric Oxide (NO) concentration increased with extract concentration, with the leaf showing the highest activity, followed by stem bark, and pulp the lowest. Although all extracts showed dose-dependent increases, none reached the gallic acid reference value.

Table 4.15 NO concentration in the leaf, pulp, and stembark extract of *Artocarpus heterophyllus* in comparison to Gallic acid.

Concentration (mg/ml)	NO concentration µg/ml			
	Leaf	Pulp	Stembark	Gallic acid
0.02	-		-	5.615 ± 0.185
10	2.289 ± 0.043 ^b	0.897 ± 0.014 ^d	1.835 ± 0.057 ^c	
20	2.581 ± 0.029 ^a	2.067 ± 0.071 ^b	2.197 ± 0.029 ^b	
40	2.772 ± 0.014 ^a	2.278 ± 0.028 ^b	2.520 ± 0.086 ^{ab}	
80	2.904 ± 0.057 ^a	2.540 ± 0.028 ^b	2.752 ± 0.071 ^a	

The result is displayed in mean ± standard deviation. Values with different superscript letters within the same row are significantly different at $p < 0.05$.

4.1.9.2 The superoxide scavenging activity (%) of ethanol extract of stem bark, leaf and pulp of *A. heterophyllus*

The superoxide scavenging activity (%) of the leaf, pulp, and stem bark extract is displayed in **Table 4.16**. The extracts showed dose-dependent superoxide scavenging activity but remained lower than gallic acid. The leaf extract was most active at lower concentration (20 mg/ml), whereas the pulp extract showed the highest activity at higher concentrations (40–80 mg/ml), with stem bark generally intermediate.

Table 4.16 Superoxide scavenging activity (%) of the leaf, pulp, and stembark extract Of *A. heterophyllus* in Comparison to Gallic acid.

Concentration (mg/ml)	% Scavenging			
	Leaf	Pulp	Stembark	Gallic acid
0.02	-		-	79.926
10	55.734	55.152	54.949	
20	65.060	60.970	58.861	
40	62.642	66.809	60.638	
80	65.179	69.451	67.253	

4.1.9.3 The Hydroxyl scavenging activity (%) of ethanol extracts of stem bark, leaf and pulp of *A. heterophyllus*

The hydroxyl scavenging activity (%) of *A. heterophyllus* (**Table 4.16**) showed that the scavenging activity increased with concentration, with leaf and stem bark extracts showing higher activity than pulp. At higher doses (40–80 mg/ml), leaf and stem bark had comparable strong activities (~71–73%), while pulp remained the least active. None exceeded gallic acid.

Table 4.17 Assessment of Hydroxyl scavenging activity (%) of *A. heterophyllus* leaf, pulp, and stembark extract in comparison to gallic acid.

Concentration (mg/ml)	% Scavenging			
	Leaf	Pulp	Stembark	Gallic acid
0.005	-		-	63.051
10	62.734	54.494	60.674	
20	67.041	56.179	63.670	
40	71.161	62.172	71.348	
80	73.034	68.727	73.221	

4.1.9.4 2,2-diphenyl-1-picrylhydrazyl (DPPH) Scavenging Activity of *A. heterophyllus*

All extracts exhibited strong scavenging 2,2-diphenyl-1-picrylhydrazyl (DPPH) activity, though none reached the BHT standard (98.50% at 0.02 mg/ml). Across all concentrations, the leaf extract consistently showed the highest scavenging activity (>93%), significantly higher than pulp and stem bark. The stem bark extract (81–86%) was intermediate, while the pulp extract (76–80%) consistently showed the lowest activity. At higher concentrations (40–80 mg/ml), the difference between leaf and the other extracts became more pronounced, confirming that the leaf possessed the strongest antioxidant potential, followed by stem bark and pulp. (Table 4.18)

Table 4.18 DPPH Scavenging Activity (%) of *Artocarpus heterophyllus* Leaf, Pulp, And Stembark Extract In Comparison To BHT.

Concentration (mg/ml)	% Scavenging			
	Leaf	Pulp	Stembark	BHT
0.02	-		-	98.501 $\pm$ 0.00
10	93.10 $\pm$ 0.18 ^a	79.838 $\pm$ 0.033 ^c	82.06 $\pm$ 0.07 ^b	
20	93.59 $\pm$ 0.07 ^a	80.405 $\pm$ 0.000 ^c	81.46 $\pm$ 0.25 ^b	
40	93.90 $\pm$ 0.00 ^a	75.966 $\pm$ 0.188 ^c	85.78 $\pm$ 0.15 ^b	
80	94.83 $\pm$ 0.00 ^a	76.581 $\pm$ 0.045 ^c	85.06 $\pm$ 0.15 ^b	

The DPPH Scavenging Activity (%) of the leaf, pulp, and stembark is presented as mean $\pm$ standard deviation. Values with different superscript letters within the same row are significantly different at $p < 0.05$.

4.1.9.5 Hydrogen Peroxide Scavenging Activity of Ethanol Extract of Stem Bark, Leaf and pulp of *A. heterophyllus*

The hydrogen peroxide Scavenging Activity (%) of the leaf, pulp, and stembark is shown in **Table 4.19**. The scavenging activity of all extracts increased with concentration, indicating a dose-dependent response. At 5 mg/ml, the leaf extract (63.00%) was the most active, followed by pulp (57.72%) and stem bark (55.60%). At 10 mg/ml, leaf activity (66.60%) remained highest and approached the gallic acid standard (69.98%), while pulp and stem bark were lower (~61%). At 50 mg/ml, the leaf extract (73.57%) matched the gallic acid standard, with pulp and stem bark showing moderate activity (~65%). At 100 mg/ml, the leaf extract peaked (76.53%), remaining the strongest, while pulp (69.35%) was moderately active, and stem bark (61.31%) was the weakest.

4.19 Hydrogen peroxide Scavenging Activity (%) of *Artocarpus heterophyllus* leaf, pulp, and stembark extract in comparison to Gallic acid.

Concentration (mg/ml)	% Scavenging			
	Leaf	Pulp	Stembark	Gallic acid
5	63.002	57.717	55.603	
10	66.596	61.099	60.888	69.979
50	73.573	65.116	65.328	73.573
100	76.533	69.345	61.311	

4.1.9.6 Ferric Reducing Antioxidant Power (FRAP) Scavenging Activity Ethanol Extract of Stem Bark, Leaf and pulp of *A. heterophyllus*

All extracts exhibited a concentration-dependent increase in Ferric Reducing Antioxidant Power (FRAP) (Table 4.20) scavenging activity, but none surpassed gallic acid at equivalent concentrations. At 10 mg/ml, stem bark (75.88%) and leaf (73.41%) were comparable to gallic acid (76.95%), while pulp was much lower (56.38%). At 20 and 40 mg/ml, stem bark extract (80.50–84.04%) showed the strongest activity, closely approaching gallic acid (82.98–85.47%), followed by leaf (75.88–80.14%) and then pulp (59.51–63.77%). At 80 mg/ml, stem bark (86.53%) maintained the highest scavenging effect, leaf (80.50%) remained moderately active, while pulp (71.17%) showed the lowest.

Table 4.20. FRAP scavenging activity (%) of *Artocarpus heterophyllus* leaf, pulp, and stembark extract in comparison to Gallic acid.

Concentration (mg/ml)	% Scavenging			
	Leaf	Pulp	Stembark	Gallic acid
10	73.406	56.38	75.882	76.946
20	75.882	59.51	80.501	82.976
40	80.141	63.77	84.041	85.465
80	80.501	71.17	86.530	94.329

4.1.9.7 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) Scavenging activity Ethanol Extract of Stem Bark, Leaf and pulp of *A. heterophyllus*

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) Scavenging activity increased with concentration for all extracts, but none reached the level of gallic acid. At 100 mg/ml, the leaf extract (67.02%) was the most active, followed by stem bark (55.18%) and pulp (52.85%), compared to 74.21% for gallic acid. At 200 mg/ml, the same trend was observed, with leaf (68.29%) higher than stem bark (57.51%) and pulp (55.18%), while gallic acid remained superior (78.86%). At 300 mg/ml, leaf activity increased further (71.25%), approaching gallic acid (82.03%), whereas stem bark (59.41%) and pulp (60.04%) remained lower.

Table 4.21. ABTS Scavenging Activity (%) Of *Artocarpus Heterophyllus* Leaf, Pulp, And Stembark Ethanol Extract in Comparison to Gallic acid.

Concentration (mg/ml)	% Scavenging			
	Leaf	Pulp	Stembark	Gallic acid
100	67.01903	52.854	55.1797	74.207
200	68.28753	55.179	57.50529	78.858
300	71.24736	60.042	59.40803	82.030

4.1.10 Acute Toxicity Study of of Ethanolic Extracts Ethanol Extract of Stem Bark, Leaf and pulp of *A. heterophyllus*

The acute toxicity study (LD₅₀) of the ethanol stem bark, leaves and pulp of *A.heterophyllus* on mice (Table 4.20) showed that no death were recorded among the test animals even at 5000mg/kg.

Table 4.22 Acute Toxicity Results Ethanol Extract of Stem Bark, Leaf and pulp of *A. heterophyllus*

Phase 1										
Plant Parts	Stem Bark			leaves				pulp		
(mg/Kg)										
Response	control	10	100	1000	10	100	1000	10	100	1000
Mortality	0/1	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Phase 2										
Response	Control	3000	4000	5000	3000	4000	5000	3000	4000	5000
Mortality	0/1	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2

LD₅₀ > 5000mg/Kg body Weight for Ethanol Extract of Stem Bark, Leaf and pulp of *A. heterophyllus*

4.1.11 Induction and confirmation of Benign Prostatic Hyperplasia (BPH).

4.1.11.1 Effect of subcutaneous testosterone propionate administration on serum PSA levels of experimental animals

Subcutaneous administration of testosterone propionate resulted in a statistically significant elevation of serum prostate-specific antigen (PSA) levels in the TP induced groups when compared to the normal control as shown in **Table 4.22**.

Table 4.23 Effect of Subcutaneous Testosterone Propionate Administration on Serum PSA Levels of Experimental Albino Rats

Groups	PSA (Before Treatment) (ng/ml)	PSA (After Treatment) (ng/ml)
1	1.34 $\square \pm \square$ 0.05 ^a	1.37 $\pm$ 0.08 ^a
2	3.04 $\square \pm \square$ 0.05 ^b	3.10 $\pm$ 0.03 ^b
3	2.98 $\square \pm \square$ 0.03 ^b	1.50 $\pm$ 0.11 ^a
4	2.98 $\square \pm \square$ 0.08 ^b	2.19 $\pm$ 0.16 ^{cd}
5	3.02 $\square \pm \square$ 0.07 ^b	2.03 $\pm$ 0.13 ^{cd}
6	2.95 $\square \pm \square$ 0.03 ^b	1.74 $\pm$ 0.43 ^{abc}
7	2.91 $\square \pm \square$ 0.05 ^b	1.53 $\pm$ 0.07 ^{ab}
8	3.01 $\square \pm \square$ 0.04 ^b	2.29 $\pm$ 0.07 ^d
9	2.95 $\square \pm \square$ 0.07 ^b	2.11 $\pm$ 0.14 ^{cde}
10	2.99 $\square \pm \square$ 0.04 ^b	1.68 $\pm$ 0.21 ^{abef}
11	2.94 $\square \pm \square$ 0.08 ^b	1.48 $\pm$ 0.04 ^{abf}

Notes: Values are expressed as mean $\pm$ SD, (n = 3). Values in the same column having different superscripts differ significantly (p < 0.05). PSA = prostatic specific antigen.

4.1.11.2 Macroscopic Evaluation of Prostate Tissues

Visual inspection of excised prostate glands revealed distinct morphological differences among the experimental groups. Group A (control) exhibited normal prostate architecture with pale coloration and compact lobes. In contrast, to B (testosterone propionate-induced) showed marked enlargement of the prostate lobes, increased vascularity, and darker coloration, consistent with hyperplastic changes.



Normal Control (A)



TP Induced (B)

Figure 4.1. Excised Prostate from Normal Contro (A) and TP induced group (B) showing visible hypertrophy, increased vascularity, and darker coloration compared to the control group (A), which retained normal architecture.

4.1.11.3 Histopathological Confirmation of Benign Prostatic HyperPlasia (BPH)

Histological examination of prostate tissues from TP-induced groups revealed epithelial hypertrophy, stromal thickening, and reduced glandular lumen space. These features were consistent with androgen-driven prostatic hyperplasia .

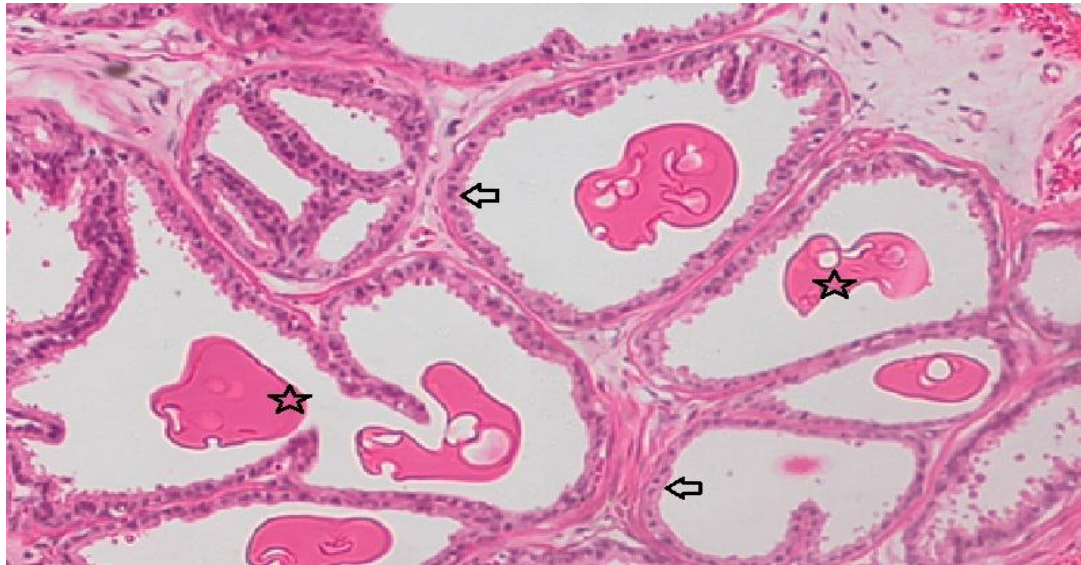


Plate 4.1 Normal Control: photomicrograph of the prostate gland showing normal histological architecture of the acini with pinkish secretions in the lumen (star) and normal epithelium of the gland (arrow). H&E. X400 magnification.

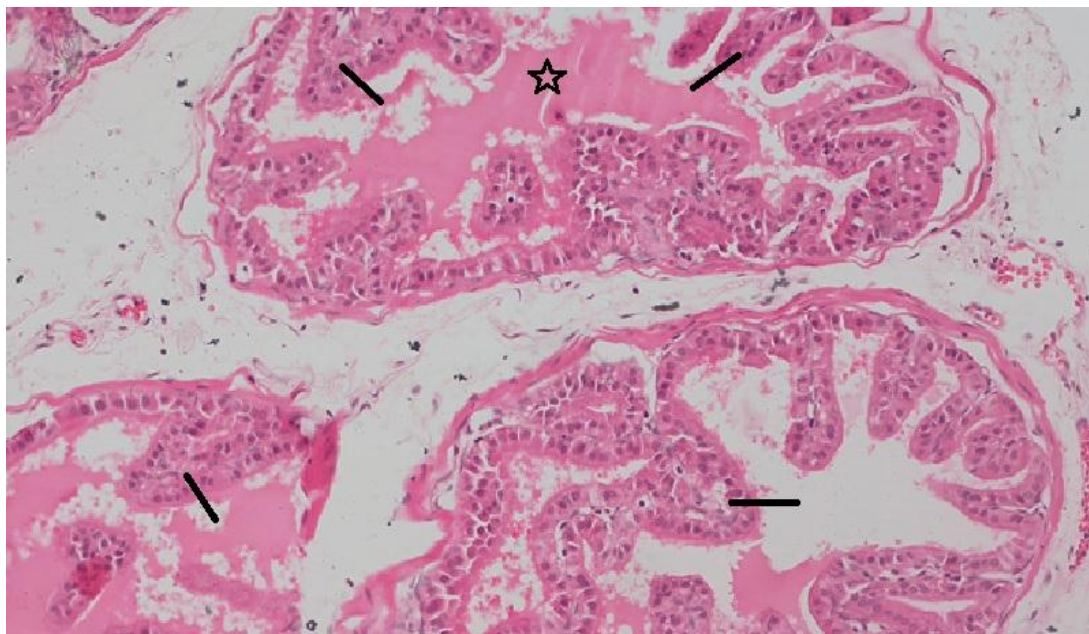


Plate 4.2 TP Induced BPH prostate. photomicrograph of the prostate gland of ratt showing the acini with pinkish secretions in the lumen (star) and thick (hypertrophied) epithelium of

the gland with projections of the epithelium into the lumen of the acini (lines) which indicated hyperplasia. X400 , stain : Hematoxylin and Eosin (H and E)

4.1.12 *In vivo* antioxidant assay of ethanolic extracts of plant parts of *A. heterophyllus*

Figure 4.2 showed the effect of ethanol extracts of plant part of *A. heterophyllus* in comparison to the Negative, positive and normal control groups, the negative exhibited the highest MDA level (3.16 ± 0.20 mmol/min/mg tissue), indicating elevated lipid peroxidation and oxidative stress. All treatment groups, including Finasteride and plant extracts, significantly reduced MDA levels to values statistically similar to the control (2.10 ± 0.36 mmol/min/mg tissue). Notably, Extract Combination group (250 mg) showed the lowest MDA (1.91 ± 0.06 mmol/min/mg tissue).

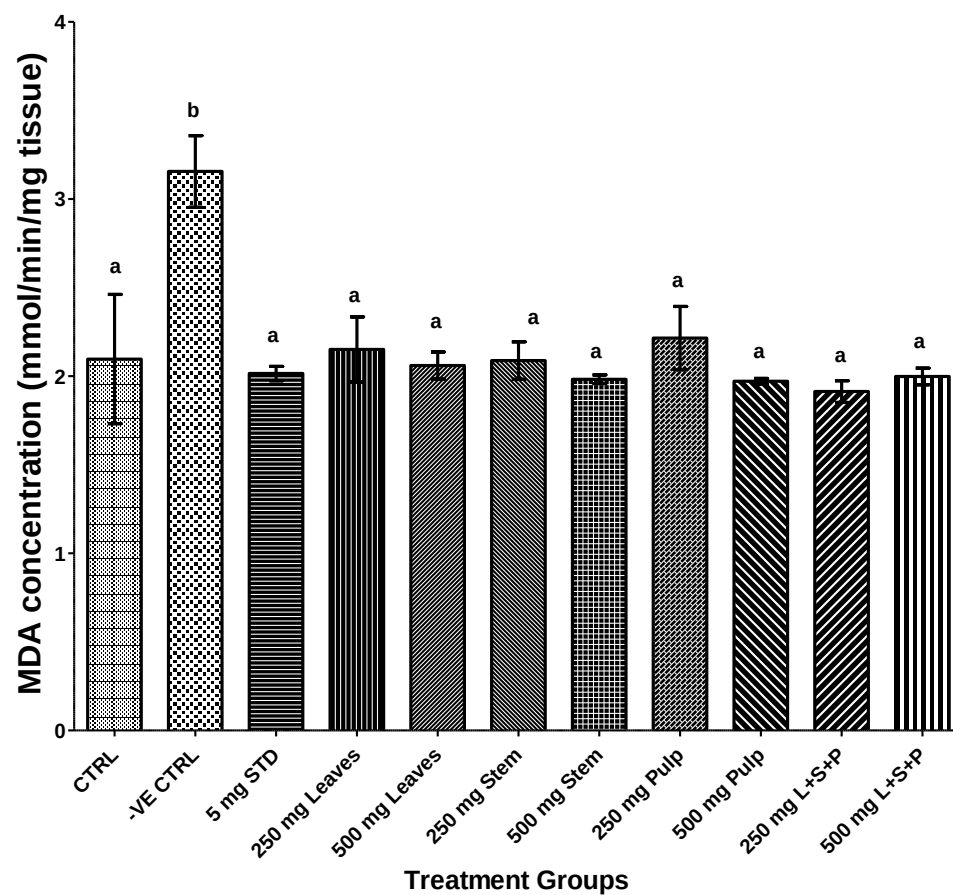


Figure. 4.2 Malondialdehyde (MDA) concentration (mmol/min/mg tissue) of rats treated with different doses of ethanolic extracts of plant parts of *A. heterophyllus*

Bars are mean $\pm$ standard deviation (n = 3). Bars bearing different alphabet letters are statistically significant ($p < 0.05$).

In figure 4.3, the Control group had the highest GSH ($12.29 \pm 0.36 \mu\text{mol/g tissue}$), while the negative control was significantly depleted (7.96 ± 0.17). Stem bark extracts (500 mg) restored GSH to near-control levels ($12.16 \pm 0.23 \mu\text{mol/g tissue}$), while Extract Combination (500 mg) also showed a marked increase ($11.29 \pm 1.31 \mu\text{mol/g tissue}$). SOD activity was severely compromised in the negative control ($19.84 \pm 1.09 \mu/\text{mg tissue}$)

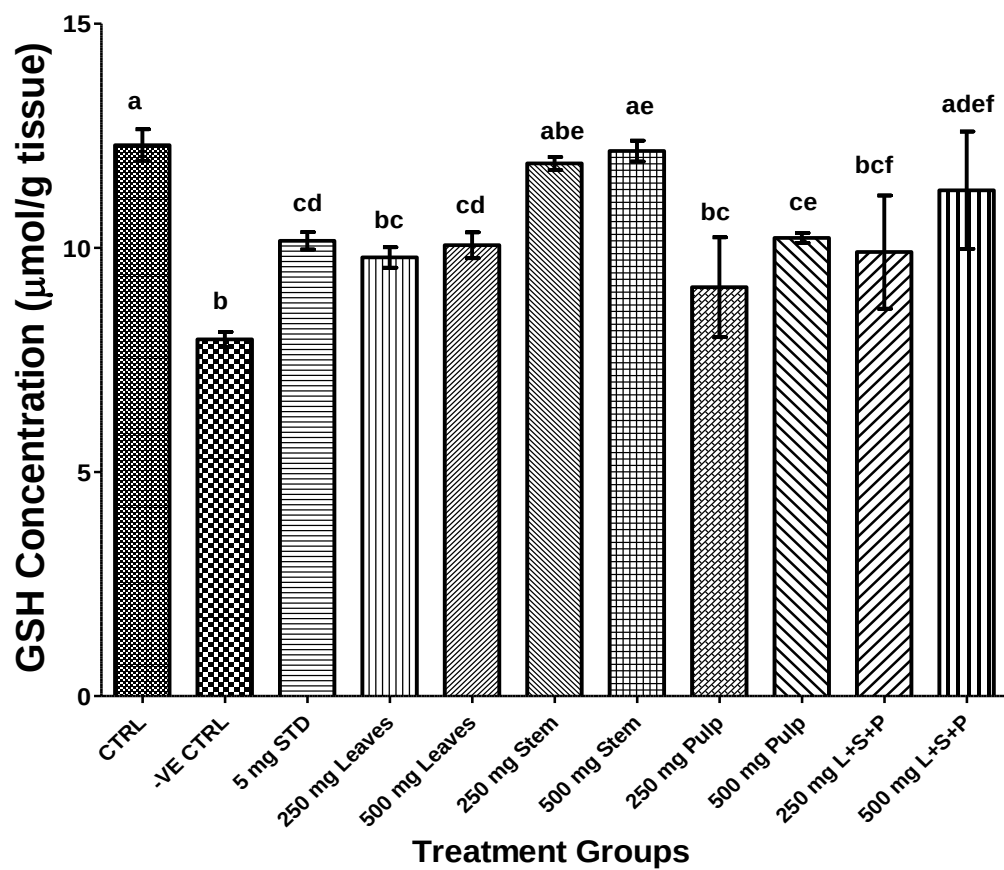


Figure. 4.3 GSH concentration ($\mu\text{mol/g}$ tissue) of Rats Treated with Different Doses of Ethanolic Extracts of Plant Parts of *A. heterophyllus*

Bars are mean $\pm$ standard deviation ($n = 3$). Bars bearing different alphabet letters are statistically significant ($p < 0.05$).

Figure 4.4, the Control maintained the highest activity ($34.88 \pm 0.24 \mu/\text{mg tissue}$), followed closely by Extract Combination (500 mg) ($33.22 \pm 1.87\mu/\text{mg tissue}$) and Stem bark (250 mg) ($31.95 \pm 0.53\mu/\text{mg tissue}$). Finasteride and other extracts showed intermediate restoration, with statistically significant improvements over the negative control

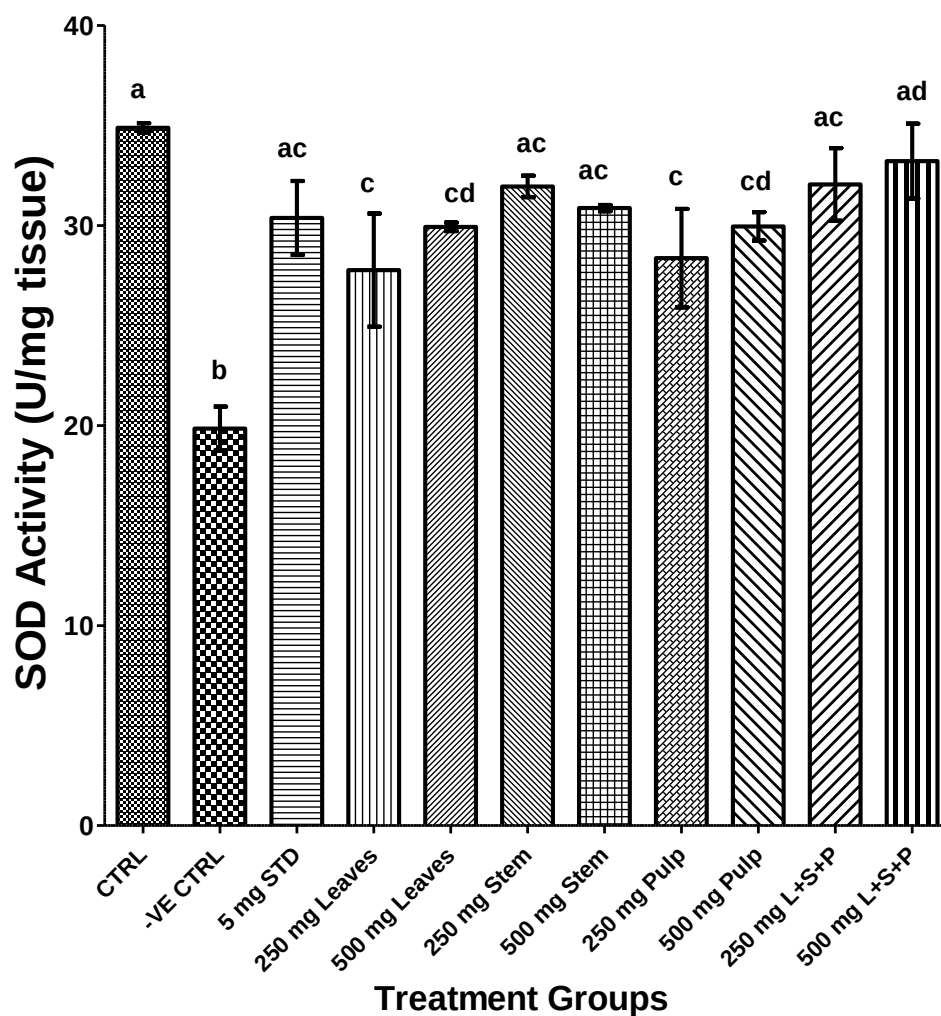


Figure. 4.4. SOD activity (μ /mg tissue) of rats treated with different doses of ethanolic extracts of plant parts of *A. heterophyllus*

Bars are mean $\pm$ standard deviation (n = 3). Bars bearing different alphabet letters are statistically significant ($p < 0.05$).

Figure 4.5 showed that catalase levels were lowest in the negative control (2.04 ± 0.11 U/min), the Control group had the highest CAT (3.34 ± 0.15 U/min) , with Extract Combination (250 mg) (3.21 ± 0.12 U/min) and Pulp (500 mg) (3.10 ± 0.06 U/min) approaching similar efficacy. Stem (500 mg) also showed strong CAT activity (3.05 ± 0.09 U/min).

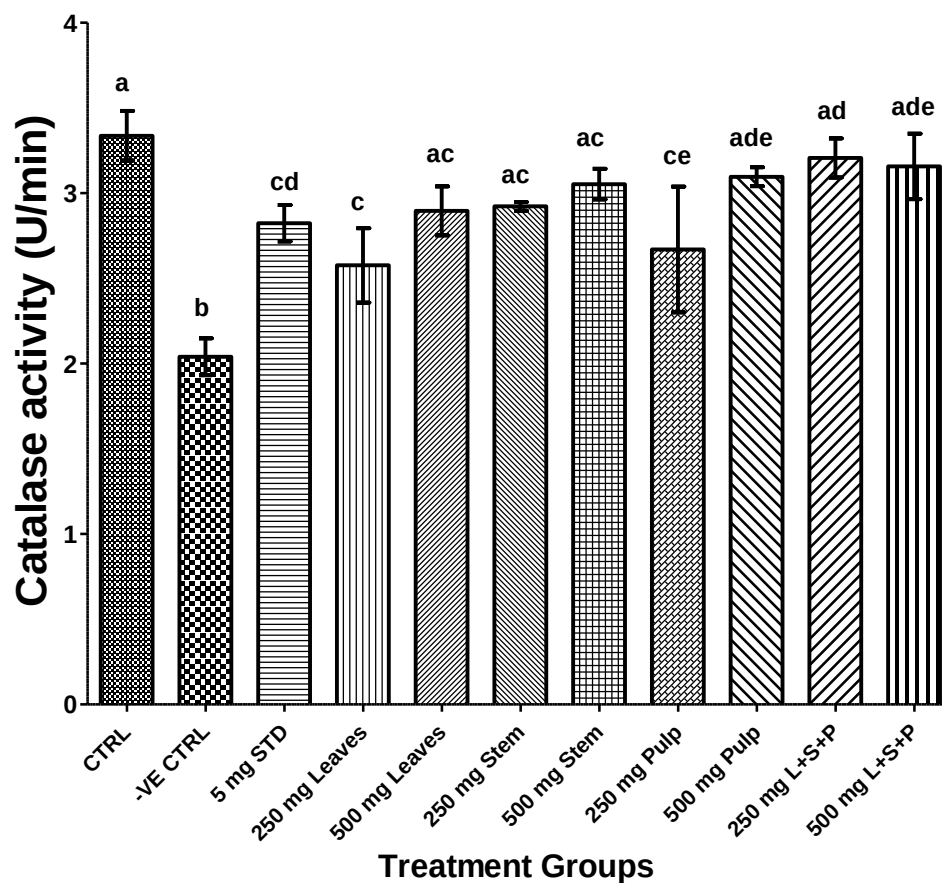


Fig. 4.5 Catalase activity (U/min) of rats treated with different doses of ethanolic extracts of plant parts of *A. heterophyllus*

Bars are mean $\pm$ standard deviation (n = 3). Bars bearing different alphabet letters are statistically significant (p<0.05).

4.1.13 Effect of plant parts ethanol extract of *A. heterophyllus* on prostate markers

In **figure 4.6**, negative control group exhibited a significant elevation (3.44 ± 0.19 ng/ml) of serum testosterone, the Control group maintained physiological testosterone (0.54 ± 0.08 ng/ml) level while Stem (500 mg) and Extract Combination (500 mg) significantly reduced testosterone to near-control levels (0.94 ± 0.11 ng/ml and 0.99 ± 0.27 ng/ml respectively), outperforming the standard drug, Finasteride (1.34 ± 0.18 ng/ml). Leaf and pulp extracts showed dose-dependent reductions, though not very pronounced.

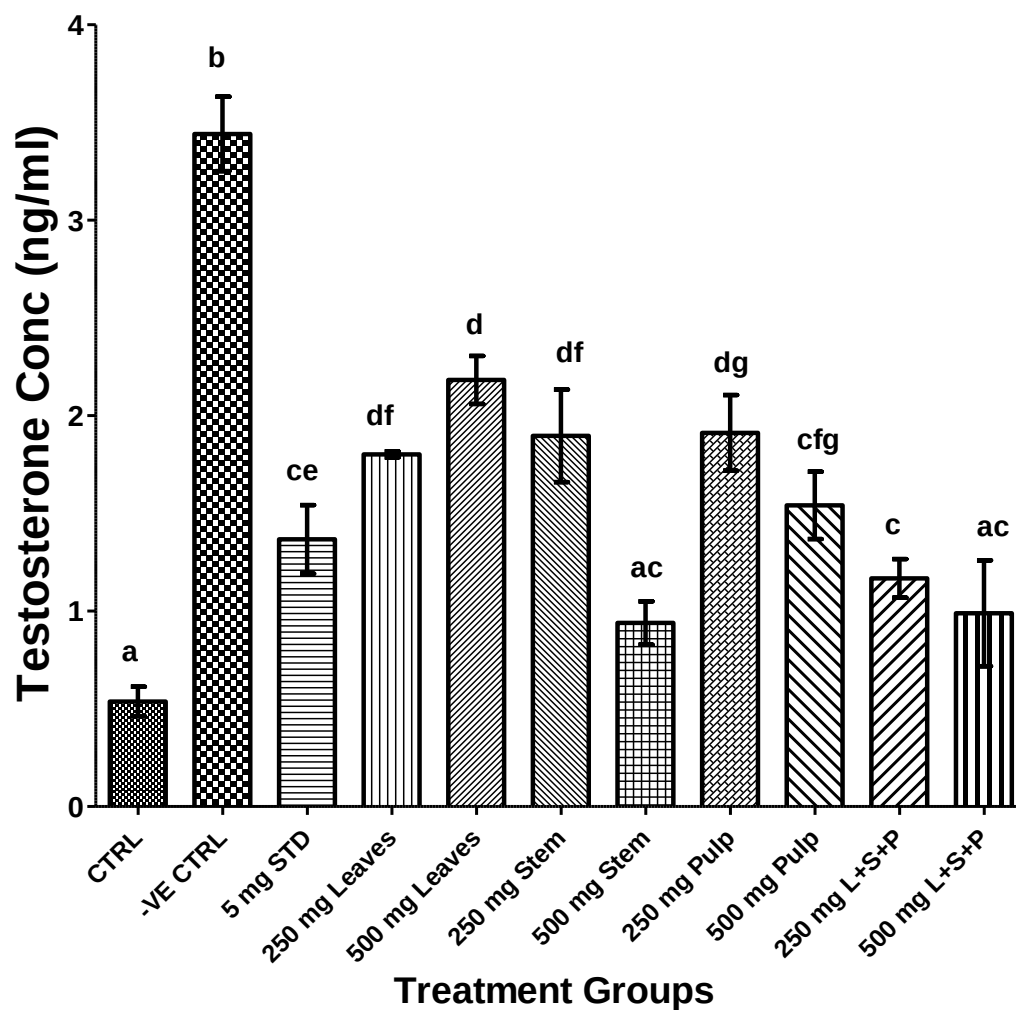


Figure. 4.6 Serum testosterone levels (ng/ml) of rats treated with different doses of ethanolic extracts of plant parts of *A. heterophyllus*

Bars are mean $\pm$ standard deviation (n = 3). Bars bearing different alphabet letters are statistically significant ($p < 0.05$).

Figure 4.7 showed that PSA was markedly elevated in the negative control (3.10 ± 0.03 ng/ml), Control and Finasteride groups maintained low PSA levels (1.37 ± 0.08 ng/ml and 1.50 ± 0.11 ng/ml) which were not significantly different. Stem (500 mg) and Ext. Combination (500 mg) restored PSA to near-control levels (1.53 ± 0.07 ng/ml and 1.48 ± 0.04 ng/ml), with no statistical difference from the standard drug.

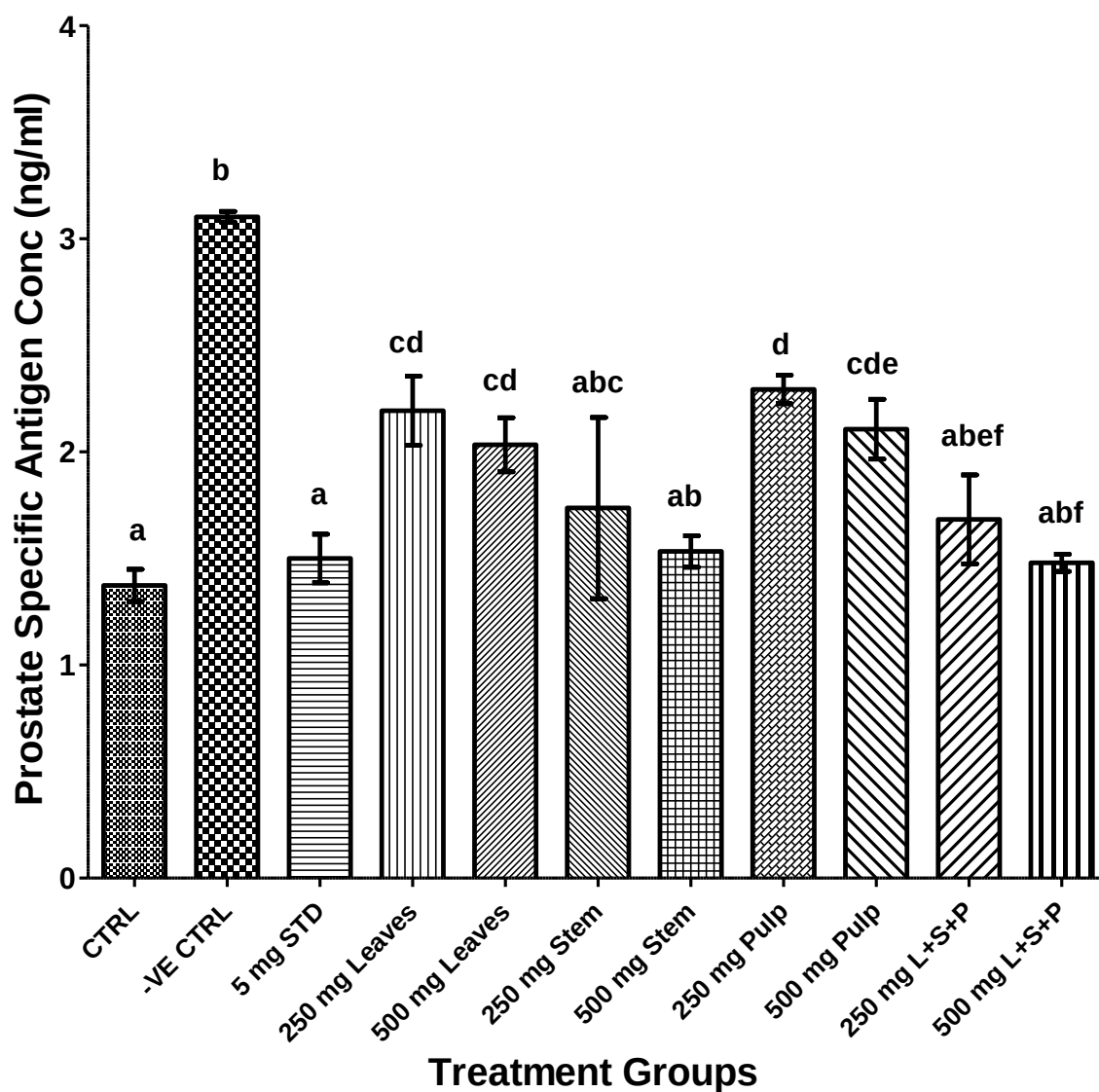


Fig. 4.7 Serum psa levels (ng/ml) of rats treated with different doses of ethanolic extracts of plant parts of *A. heterophyllus*

Bars are mean $\pm$ standard deviation (n = 3). Bars bearing different alphabet letters are statistically significant ($p < 0.05$).

TNF levels were significantly elevated in the negative control (25.22 ± 0.89), as shown in **figure 4.8**, Control group had baseline TNF (16.23 ± 0.35 pg/ml). Extract Combination (500 mg) and Finasteride significantly reduced TNF (17.25 ± 0.31 pg/ml and 17.64 ± 0.11 pg/ml) . Other extracts showed moderate reductions, with stem and leaf extracts tending toward significance.

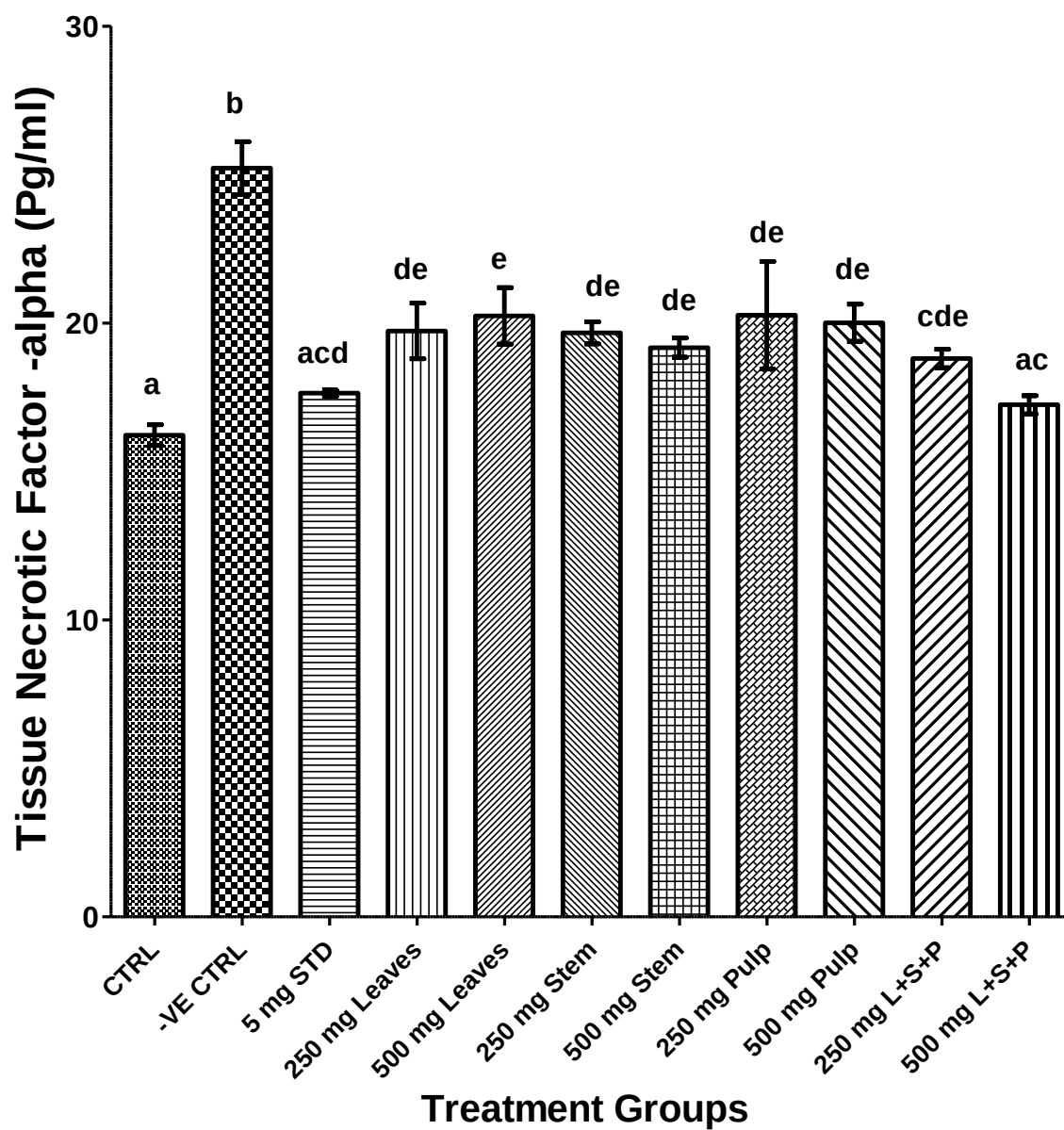


Fig. 4.8 Serum TNF levels (pg/ml) of rats treated with different doses of ethanolic extracts of plant parts of *A. heterophyllus*

Bars are mean $\pm$ standard deviation (n = 3). Bars bearing different alphabet letters are statistically significant ($p < 0.05$).

Figure 4.9 showed Interleukin levels peaked in the negative control (85.30 ± 0.39 pg/ml), Control group maintained physiological levels (53.20 ± 1.78 pg/ml). Extract Combination (500 mg) and Stem (500 mg) restored interleukin levels to near-control values (59.02 ± 1.27 and 60.25 ± 2.87), outperforming other extract groups. Finasteride showed moderate suppression (61.75 ± 2.35 pg/ml).

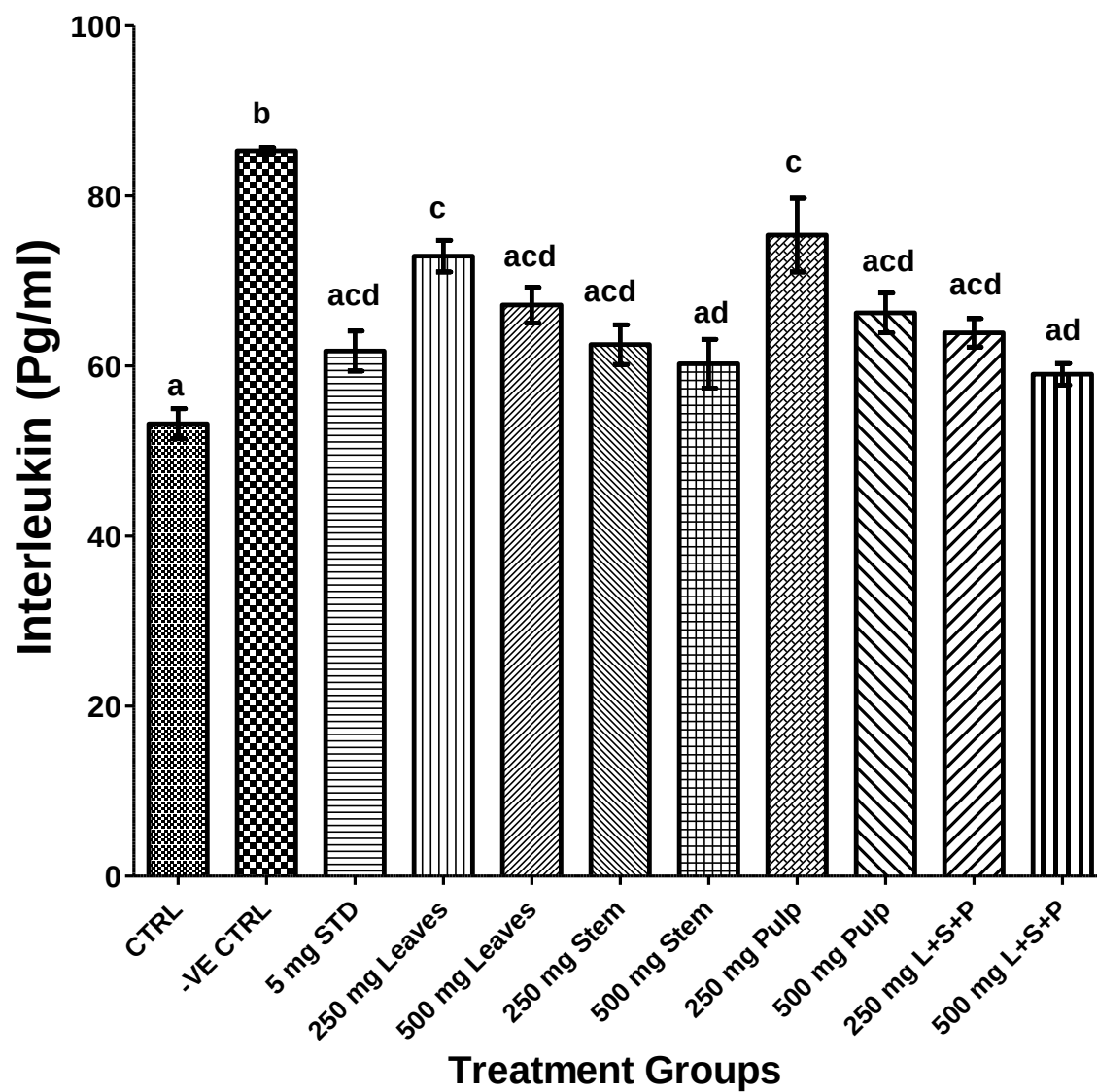


Fig. 4.9 Serum interleukin concentration (pg/ml) of rats treated with different doses of ethanolic extracts of plant parts of *A. heterophyllus*

Bars are mean $\pm$ standard deviation (n = 3). Bars bearing different alphabet letters are statistically significant ($p < 0.05$).

4.1.14 Effect of plant parts extract of *A. heterophyllus* on hepatic parameters

From **figure 4.10**, the negative control group showed significantly elevated serum ALT (22.67 ± 0.62 U/L), Control group maintained physiological ALT (15.14 ± 0.30 U/L) value. The Stem (500 mg) and Extract Combination (500 mg) restored ALT levels to near-control values (15.16 ± 0.22 U/L and 14.50 ± 0.37 U/L respectively), with no statistical difference from control. Finasteride and leaf/pulp extracts showed moderate reductions but remained significantly higher ($p < 0.005$) than control.

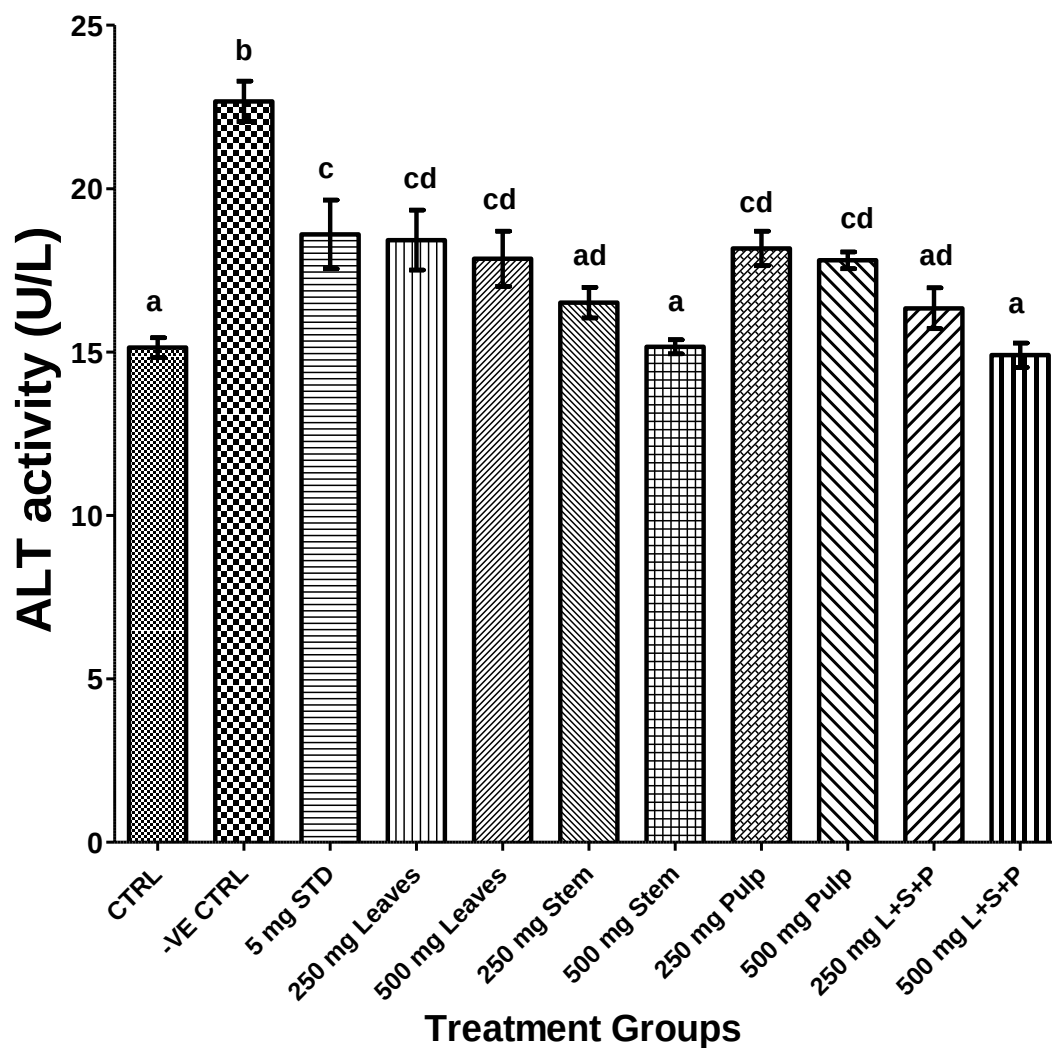


Figure. 4.10 Serum ALT activity (U/L) of rats treated with different doses of ethanolic extracts of plant parts of *A. heterophyllus*

Bars are mean $\pm$ standard deviation (n = 3). Bars bearing different alphabet letters are statistically significant ($p < 0.05$).

AST was markedly elevated in the negative control (23.97 ± 2.28 U/L) as observed in **figure 4.11**, Control group had baseline serum AST level (13.15 ± 1.05 U/L). while Stem (500 mg) and Ext. Combination (500 mg) significantly reduced AST (12.88 ± 0.15 U/L and 13.90 ± 0.54 U/L), statistically indistinguishable from control ($p < 0.05$). Other treatments showed partial reductions, with leaf and pulp extracts remaining elevated when compared to the normal control.

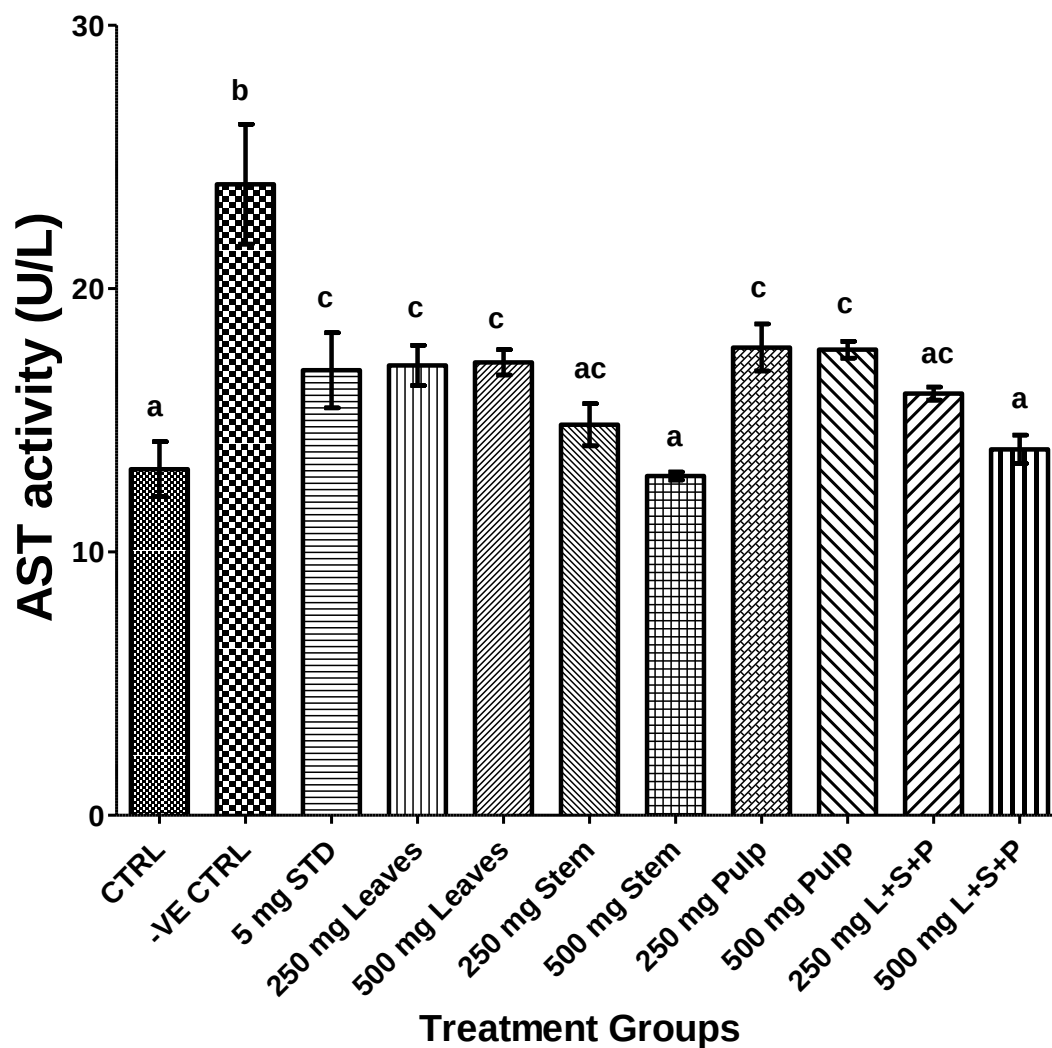


Figure. 4.11 Serum AST activity (u/l) of rats treated with different doses of ethanolic extracts of plant parts of *A. heterophyllus*

Bars are mean $\pm$ standard deviation (n = 3). Bars bearing different alphabet letters are statistically significant ($p < 0.05$).

Serum ALP was highest in the negative control (179.50 ± 6.96 U/L), **figure 4.12** , Control group maintained normal ALP (138.60 ± 6.97) levels while, Stem (500 mg) and Extract Combination (500 mg) showed significant reductions (129.30 ± 0.94 U/L and 135.90 ± 1.28 U/L) , statistically similar to control ($p < 0.05$). Leaf and pulp extracts showed intermediate reductions, with Leaves (250 mg) remaining significantly elevated (158.40 ± 2.86 U/L).

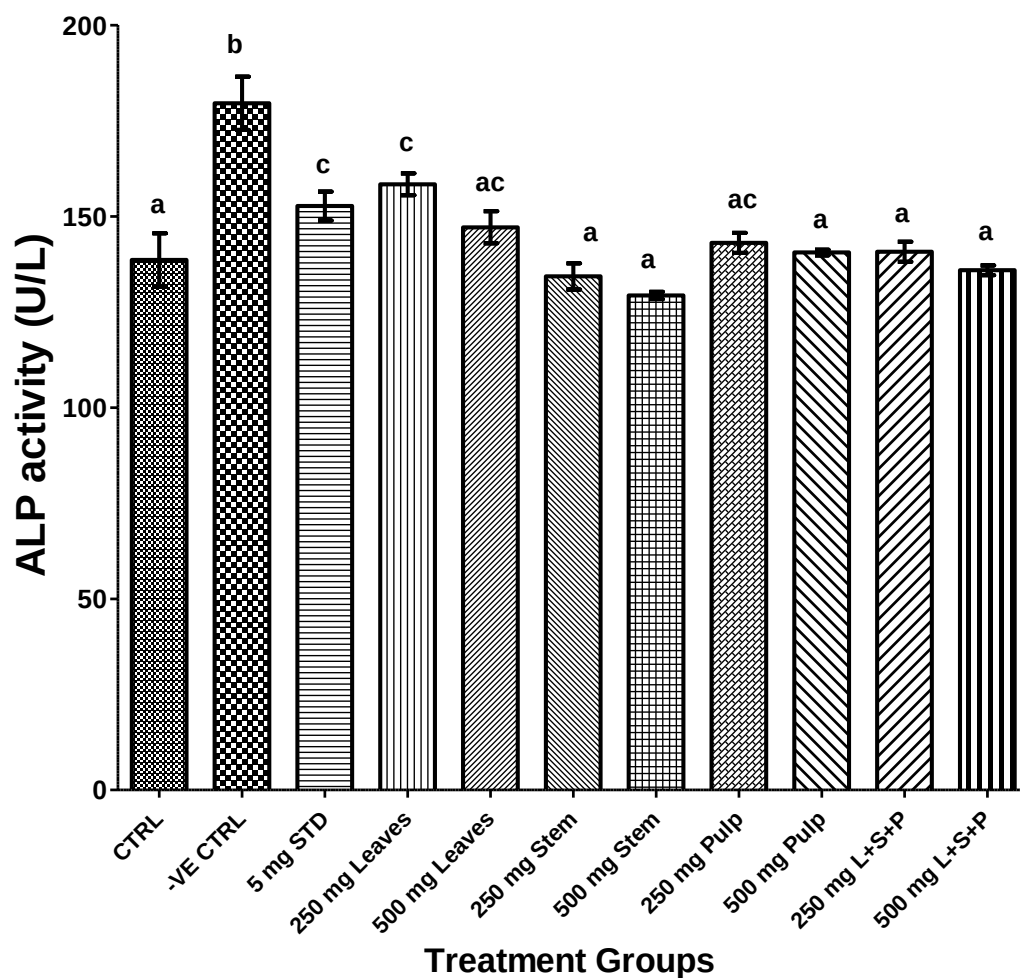


Figure. 4.12 Serum ALP activity (U/L) of rats treated with different doses of ethanolic extracts of plant parts of *A. heterophyllus*

Bars are mean $\pm$ standard deviation (n = 3). Bars bearing different alphabet letters are statistically significant (p<0.05).

In **figure 4.13** LDH levels were slightly elevated in the negative control (132.80 ± 3.06), though not statistically different from control (136.40 ± 4.35 U/L) ($p < 0.05$). Leaves (500 mg) and Stem (500 mg) showed significant increases (163.20 ± 7.16 U/L and 159.40 ± 0.76 U/L), Ext. Combination (500 mg) maintained LDH within normal range (138.10 ± 2.63 U/L).

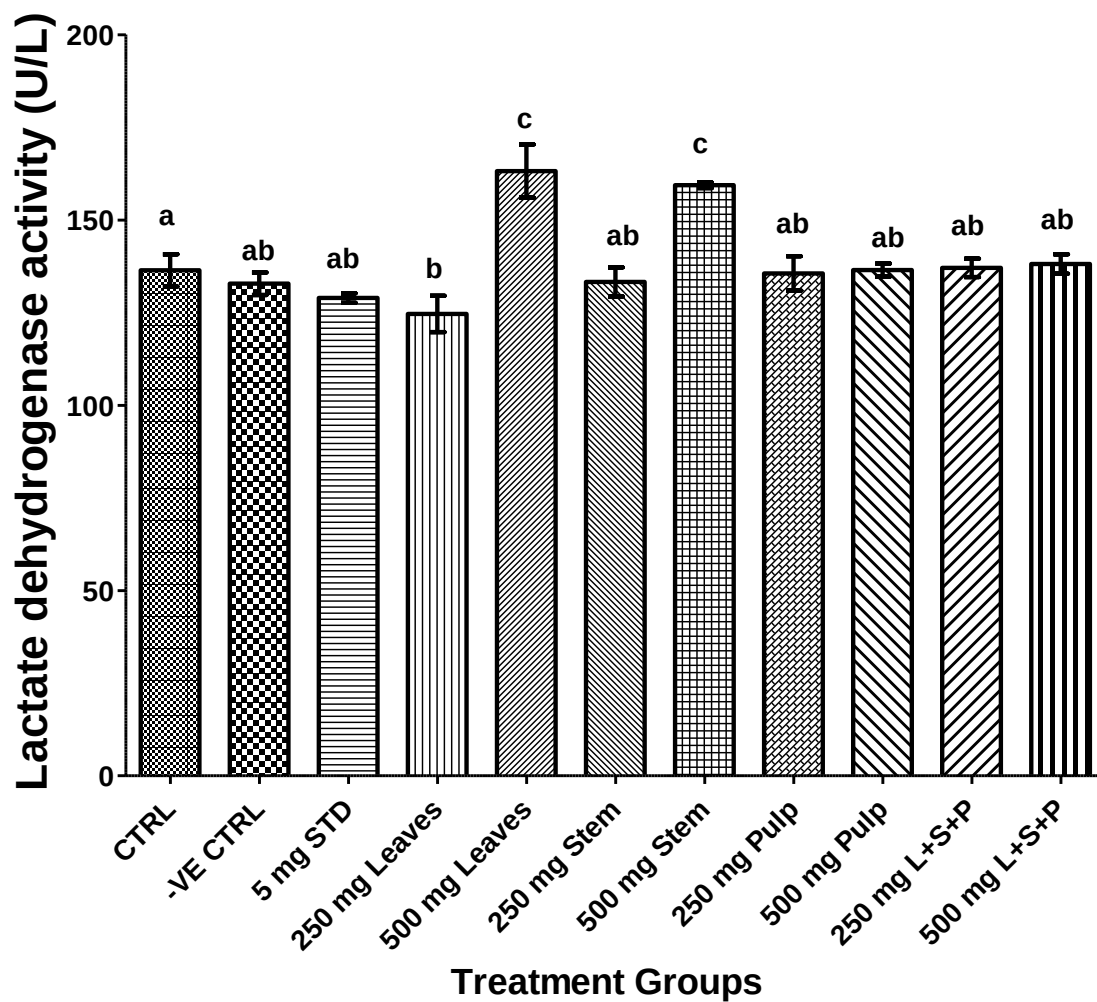


Figure. 4.13 Serum LDH activity (U/L) of rats treated with different doses of ethanolic extracts of plant parts of *A. heterophyllus*

Bars are mean $\pm$ standard deviation (n = 3). Bars bearing different alphabet letters are statistically significant (p<0.05).

4.1.15 Effect of plant parts extract of *A. heterophyllus* on liver protein and bilirubin concentrations

It was observed in **figure 4.14** that the Negative control group exhibited significantly elevated bilirubin ($24.40 \pm 0.93 \mu\text{mol/L}$), Control group maintained physiological levels ($16.06 \pm 0.54 \mu\text{mol/L}$) with the Stem (500 mg) and Ext. Combination (500 mg) restored bilirubin levels to near-control values ($15.31 \pm 9.32 \mu\text{mol/L}$ and $16.50 \pm 0.16 \mu\text{mol/L}$), with no statistical difference from control ($p < 0.05$). Leaf and pulp extracts showed moderate reductions but remained significantly elevated.

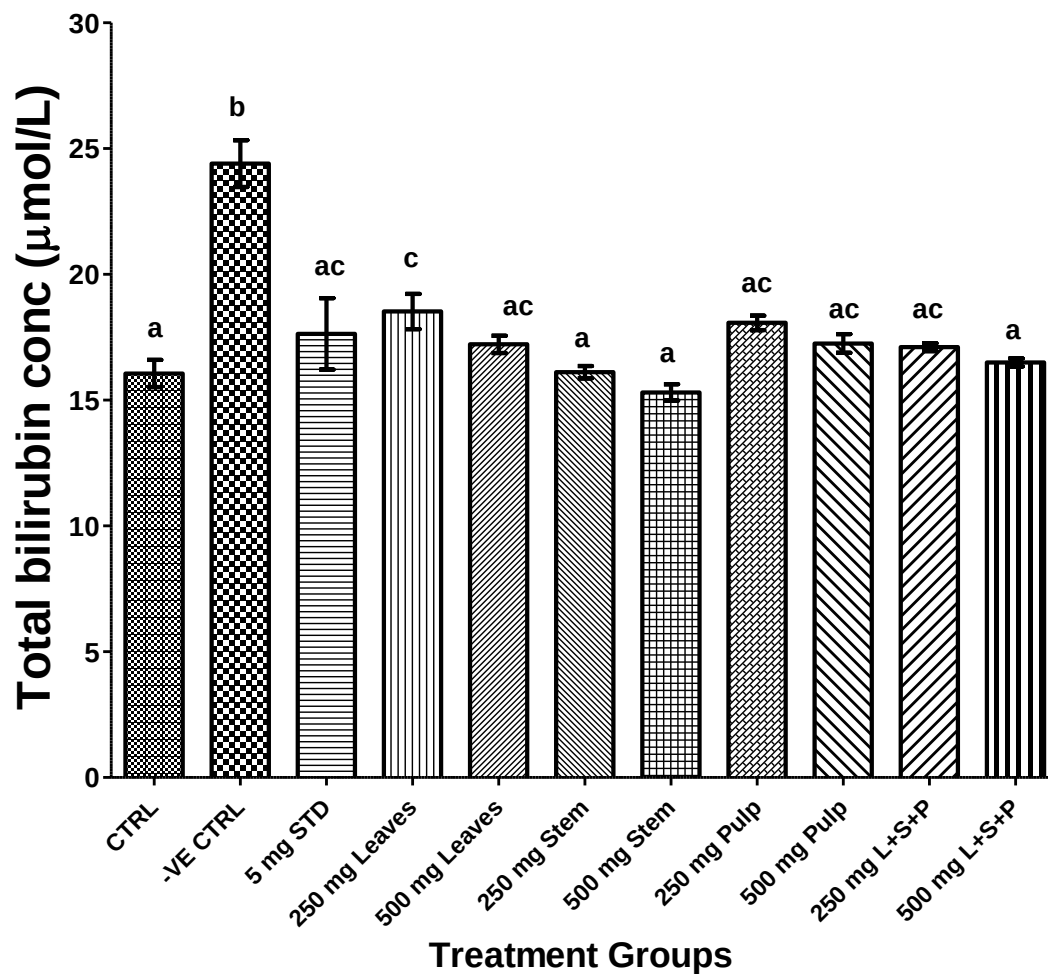


Figure. 4.14 Serum total bilirubin concentration ($\mu\text{mol/L}$) of rats treated with different doses of ethanolic extracts of plant parts of *A. heterophyllus*

Bars are mean $\pm$ standard deviation ($n = 3$). Bars bearing different alphabet letters are statistically significant ($p < 0.05$).

Direct bilirubin (**figure 4.15**) was highest in the negative control ($17.21 \pm 0.60 \mu\text{mol/L}$), Control group had baseline levels ($11.76 \pm 0.33 \mu\text{mol/L}$) while it was observed that Stem (500 mg) and Ext. Combination (500 mg) significantly reduced direct bilirubin ($12.54 \pm 0.52 \mu\text{mol/L}$ and $13.03 \pm 0.09 \mu\text{mol/L}$), statistically similar to control ($p < 0.05$). Leaf and pulp extracts showed partial improvement but remained elevated.

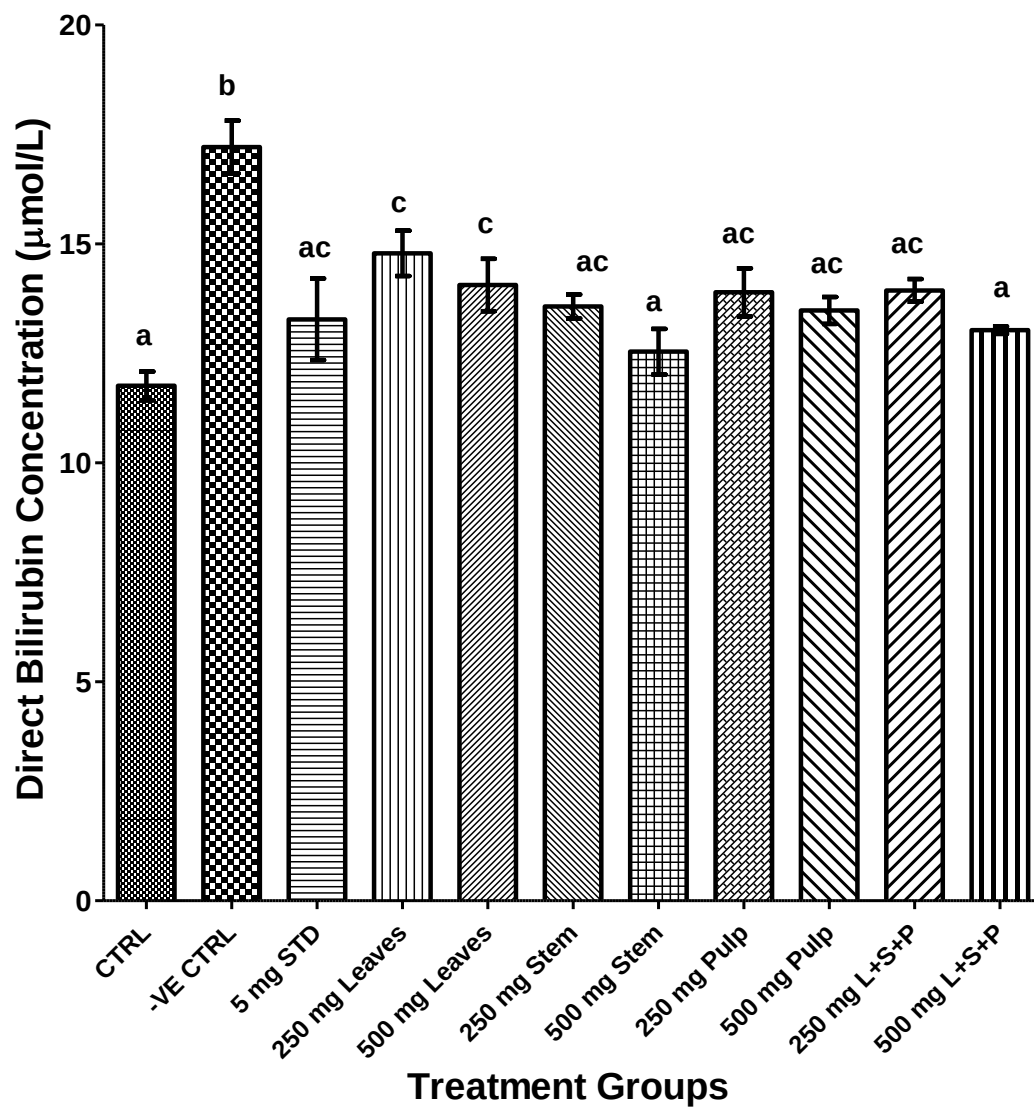


Figure. 4.15 Serum direct bilirubin concentration ($\mu\text{mol/L}$) of rats treated with different doses of ethanolic extracts of plant parts of *A. heterophyllus*

Bars are mean $\pm$ standard deviation ($n = 3$). Bars bearing different alphabet letters are statistically significant ($p < 0.05$).

In **figure 4.16**, the negative control group showed marked hypoproteinemia (5.29 ± 0.85 mg/L), the Control group maintained normal protein levels (10.00 ± 0.37 mg/L), Stem (500 mg) and Finasteride significantly restored protein levels (9.39 ± 0.16 mg/L and 8.74 ± 0.21 mg/L) ($p < 0.05$), approaching control values. Other extracts showed moderate improvements, with leaf and pulp extracts remaining suboptimal.

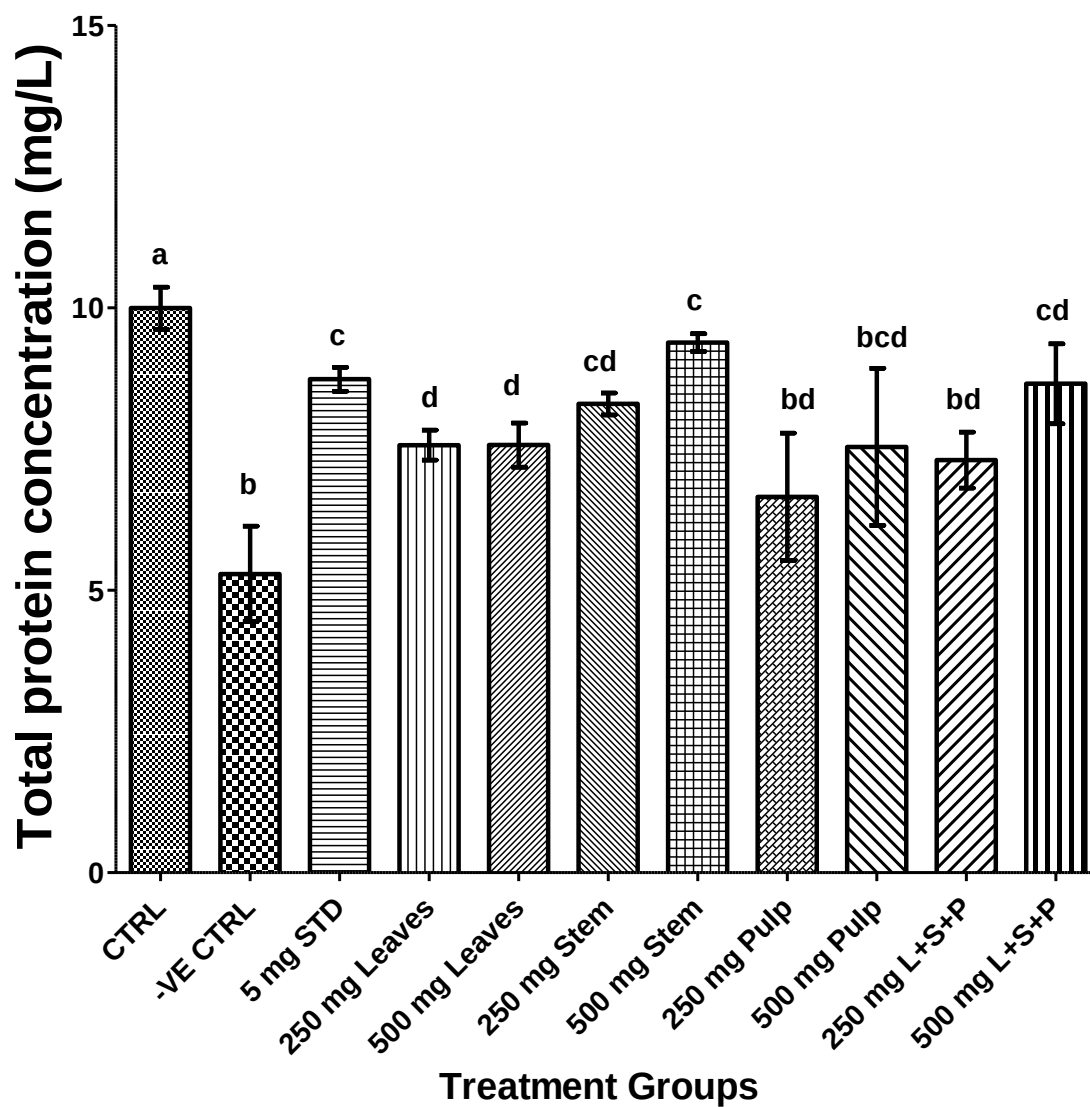


Figure. 4.16 Serum total protein concentration (mg/L) of rats treated with different doses of ethanolic extracts of plant parts of *A. heterophyllus*

Bars are mean $\pm$ standard deviation (n = 3). Bars bearing different alphabet letters are statistically significant ($p < 0.05$).

figure 4.17. shows Albumin was significantly reduced in the negative control (3.76 ± 0.54 mg/L), when compared to the control group ($p < 0.05$) which maintained normal albumin (5.81 ± 0.35 mg/L). Stem (250 mg & 500 mg) and Leaves (500 mg) showed significant restoration (5.29 ± 0.24 , 5.19 mg/L ± 0.05 , and 4.98 ± 0.65 mg/L respectively). Ext. Combination (500 mg) also improved albumin (4.88 ± 0.62 mg/L), though slightly below control.

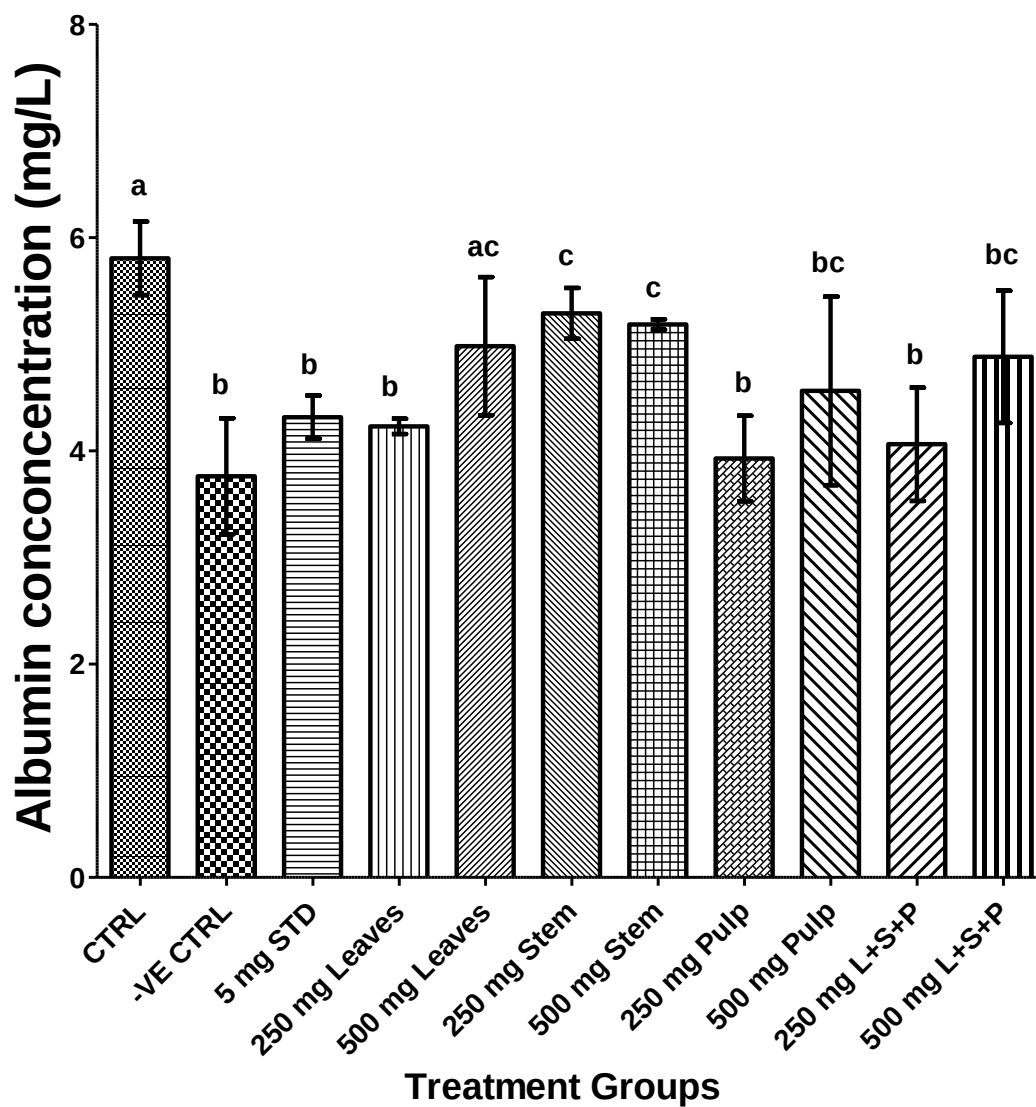


Figure. 4.17 Serum total albumin concentration (mg/L) of rats treated with different doses of ethanolic extracts of plant parts of *A. heterophyllus*

Bars are mean $\pm$ standard deviation (n = 3). Bars bearing different alphabet letters are statistically significant ($p < 0.05$).

Globulin was lowest in the negative control (1.88 ± 0.19) as seen in **figure 4.18**, Control group had healthy globulin levels (4.19 ± 0.15 mg/L). Finasteride and Stem (500 mg) restored globulin to near-control levels (4.42 ± 0.40 mg/L and 4.20 ± 0.17 mg/L) Leaf and pulp extracts showed variable recovery, with Leaves (500 mg) remaining significantly ($p < 0.05$) lower (2.59 ± 0.38 mg/L) than the control level.

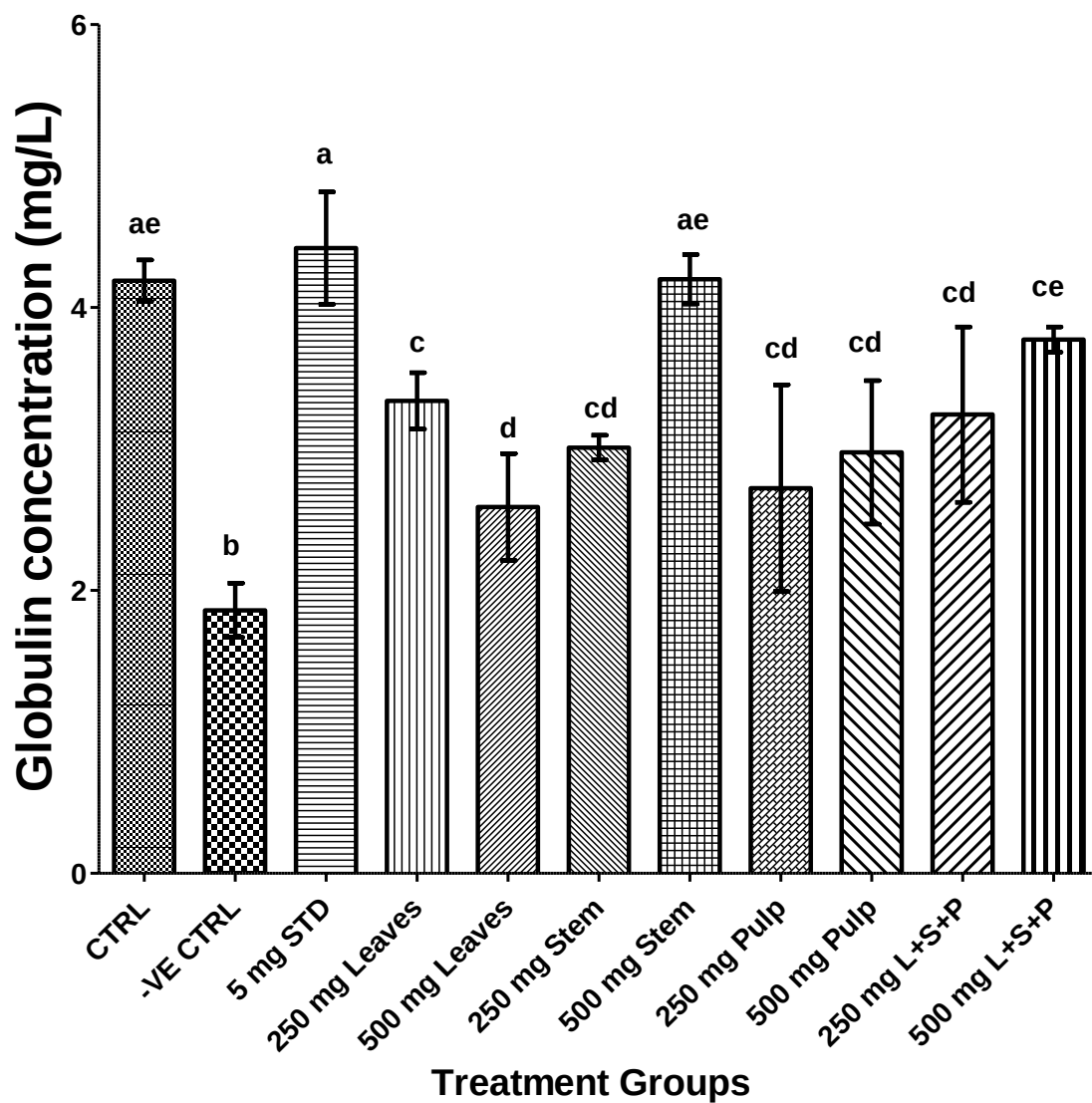


Figure. 4.18 Serum total globulin concentration. (mg/L) of rats treated with different doses of ethanolic extracts of plant parts of *A. Heterophyllus*

Bars are mean $\pm$ standard deviation (n = 3). Bars bearing different alphabet letters are statistically significant ($p < 0.05$).

4.1.16 Effect of plant parts extract of *A. heterophyllus* on renal function parameters

Negative control group showed a statistically significant ($p < 0.05$) serum urea elevation (30.43 ± 1.98 mg/dL) in **figure 4.19**, Control group maintained physiological levels (18.11 ± 0.26 mg/dL). Finasteride and Stem (250 mg) showed near-normal urea levels (19.39 ± 1.04 mg/dL and 21.82 ± 0.76 mg/dL), Extract Combination (250 mg) also showed improvement (21.17 ± 0.38 mg/dL), while Leaves (250 mg) and Pulp (250 mg) remained significantly elevated.

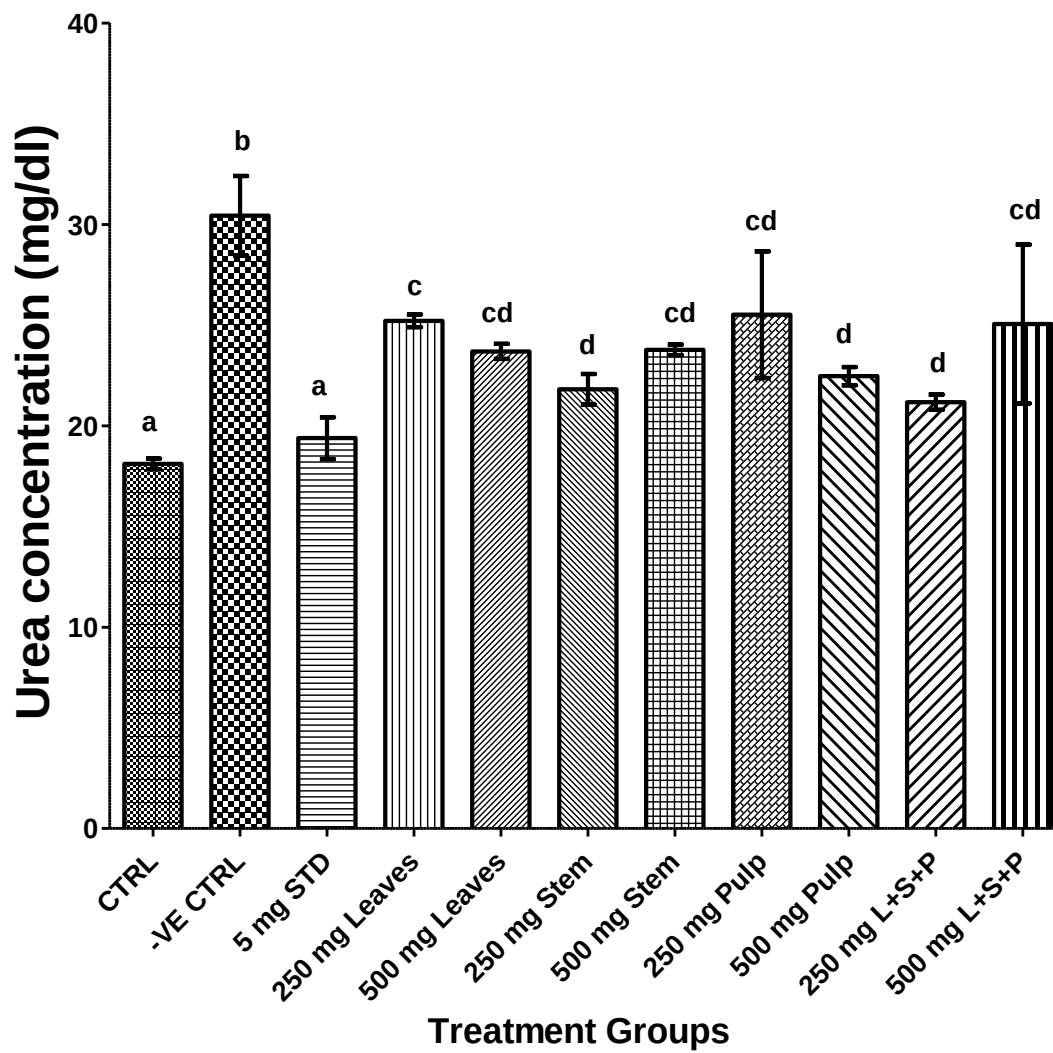


Figure. 4.19 Serum urea concentration (mg/dL) of rats treated with different doses of ethanolic extracts of plant parts of *A. heterophyllus*

Bars are mean $\pm$ standard deviation (n = 3). Bars bearing different alphabet letters are statistically significant ($p < 0.05$).

Creatinine was highest in the negative control (7.27 ± 1.01 mg/dL) as seen in **figure 4.20**, Control and Finasteride groups maintained normal levels (4.60 ± 0.25 and 4.49 ± 0.14). Stem (250 mg) and Ext. Combination (250 mg) showed creatinine levels statistically similar to control (4.66 ± 0.17 mg/dL and 4.72 ± 0.21 mg/dL). Ext. Combination (500 mg) showed elevated creatinine (6.01 ± 0.62 mg/dL).

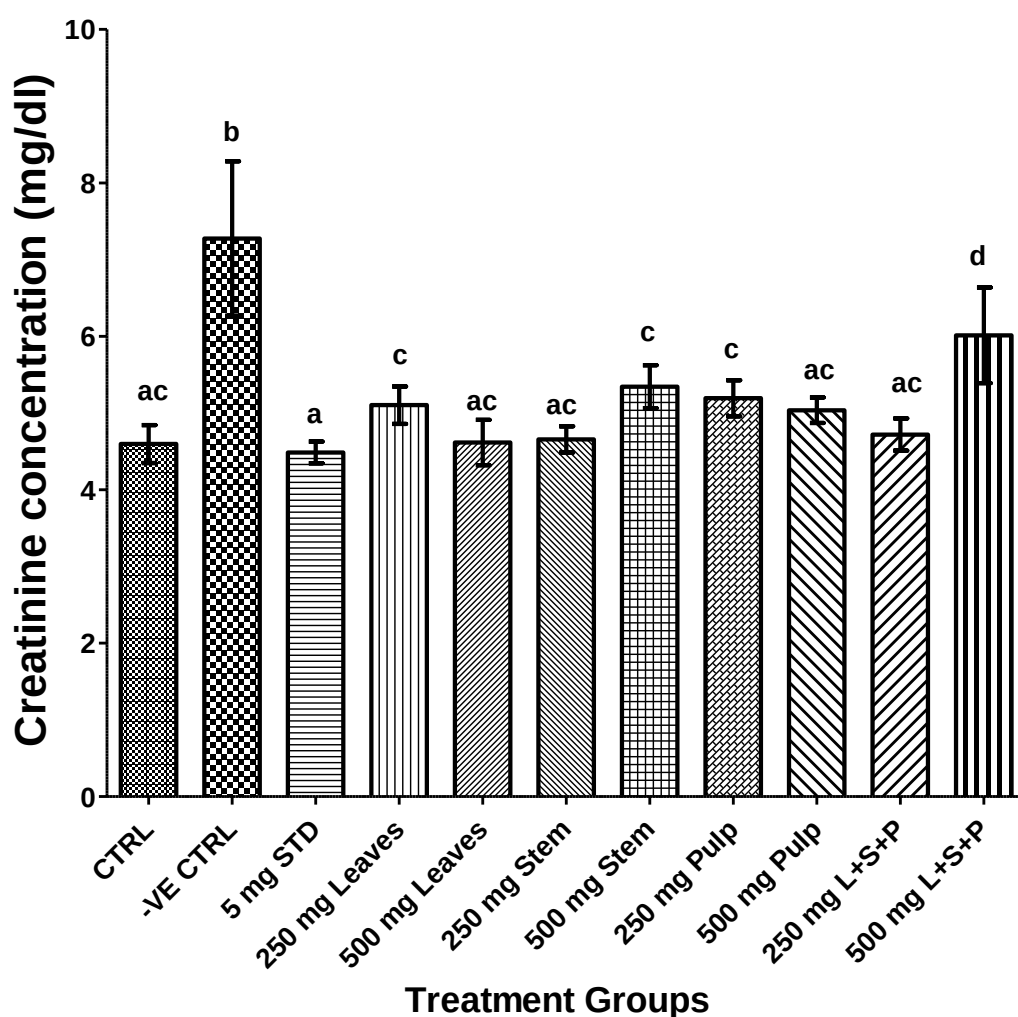


Figure. 4.20 Serum creatinine concentration (mg/dL) of rats treated with different doses of ethanolic extracts of plant parts of *A. heterophyllus*

Bars are mean $\pm$ standard deviation (n = 3). Bars bearing different alphabet letters are statistically significant ($p < 0.05$).

Figure 4.21 showed Sodium levels were significantly elevated in the negative control (187.20 ± 5.65 mmol/L). All treatment groups, including Finasteride, maintained sodium levels statistically similar to control (169.00 ± 3.66 mmol/L), ranging between 165.40–175.80 mmol/L.

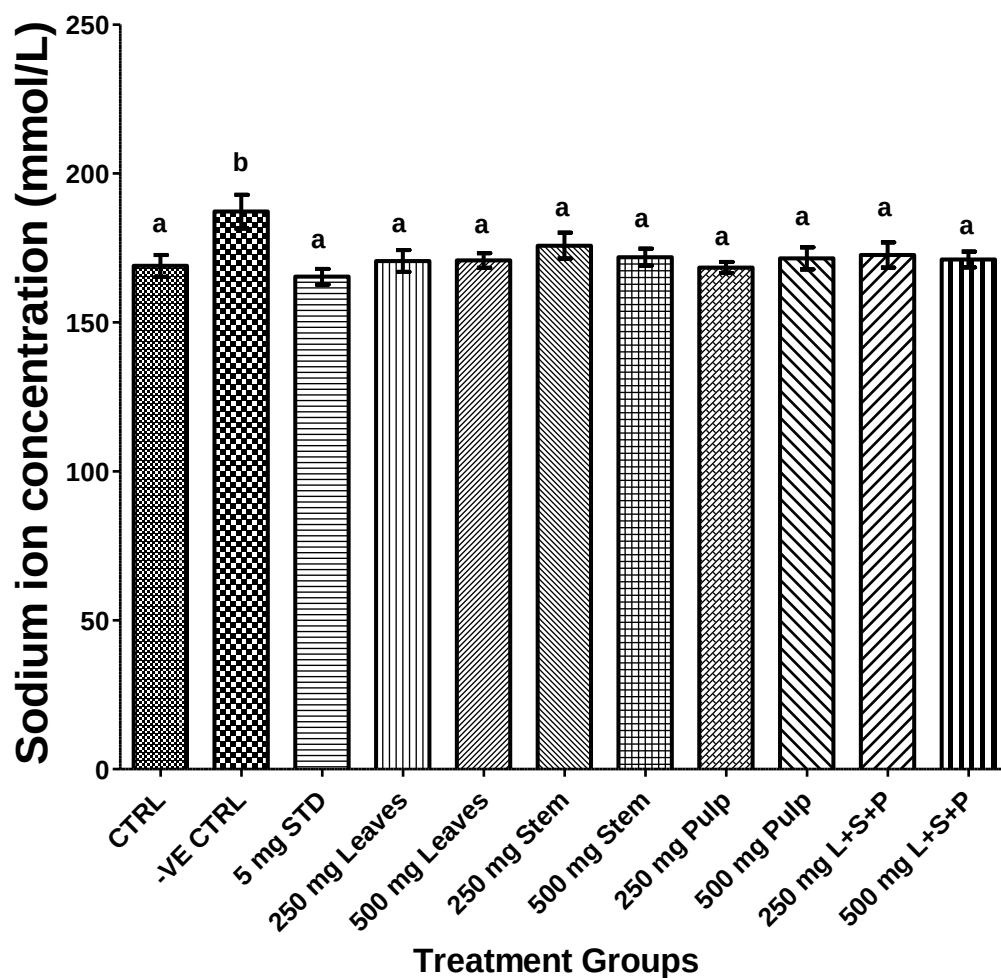


Figure. 4.21 Serum sodium ion concentration (mmol/L) of rats treated with different doses of ethanolic extracts of plant parts of *A. heterophyllus*

Bars are mean $\pm$ standard deviation (n = 3). Bars bearing different alphabet letters are statistically significant (p<0.05).

Potassium levels remained relatively stable across all groups, with no statistically significant deviations as seen in **Figure 4.22** , Ext. Combination (500 mg) showed the highest potassium (5.80 ± 0.60 mmol/L), slightly above control (5.33 ± 0.10 mmol/L).

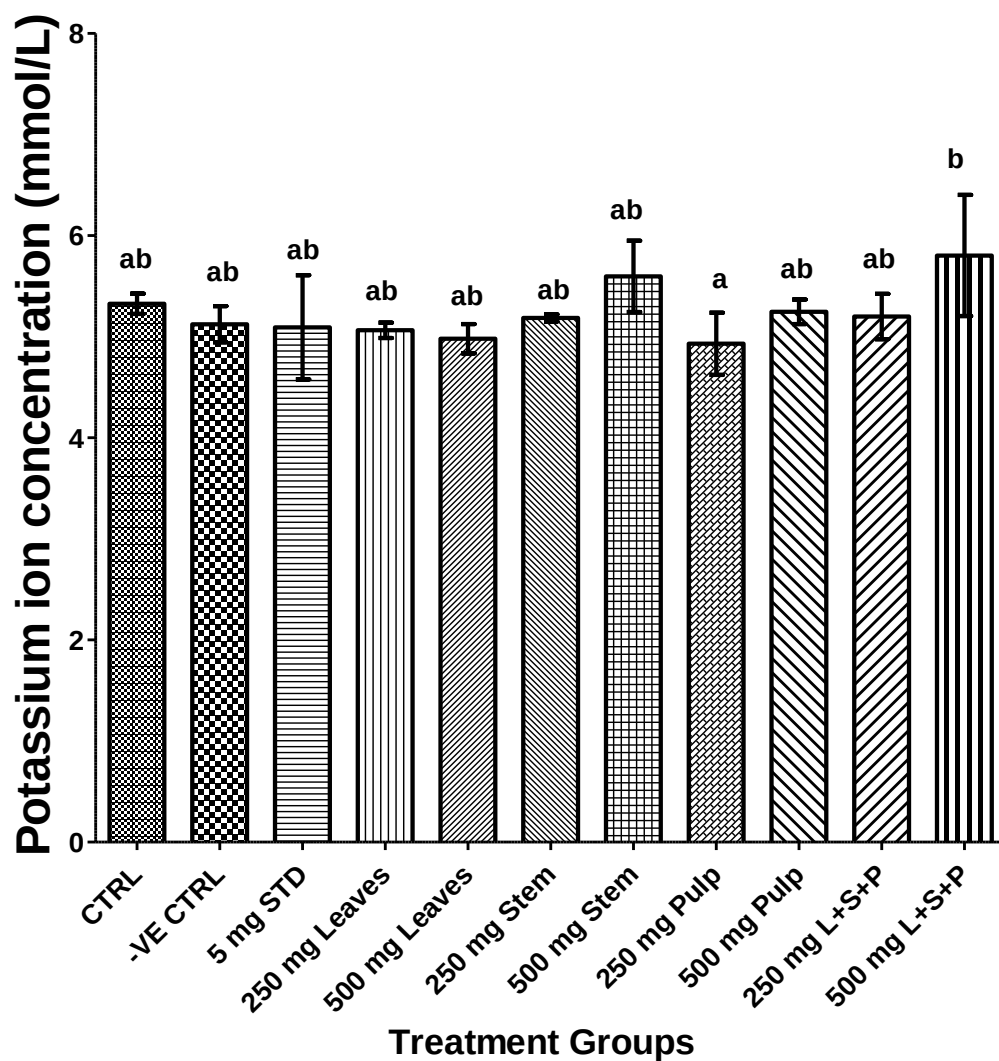


Figure. 4.22 Serum potassium ion concentration (mmol/L) of rats treated with different doses of ethanolic extracts of plant parts of *A. heterophyllus*

Bars are mean $\pm$ standard deviation (n = 3). Bars bearing different alphabet letters are statistically significant ($p < 0.05$).

Negative control showed significant depletion (14.34 ± 1.17 mmol/L) in serum bicarbonate level as observed in **Figure 4.23**, Control group maintained normal bicarbonate (20.50 ± 2.58 mmol/L) level while Ext. Combination (500 mg) restored bicarbonate to control levels (20.71 ± 0.17 mmol/L), outperforming Finasteride (17.66 ± 0.69 mmol/L). Stem (250 mg) and Ext. Combination (250 mg) also showed strong recovery (18.13 ± 0.31 mmol/L and 18.90 ± 0.14 mmol/L) when compared to the control group.

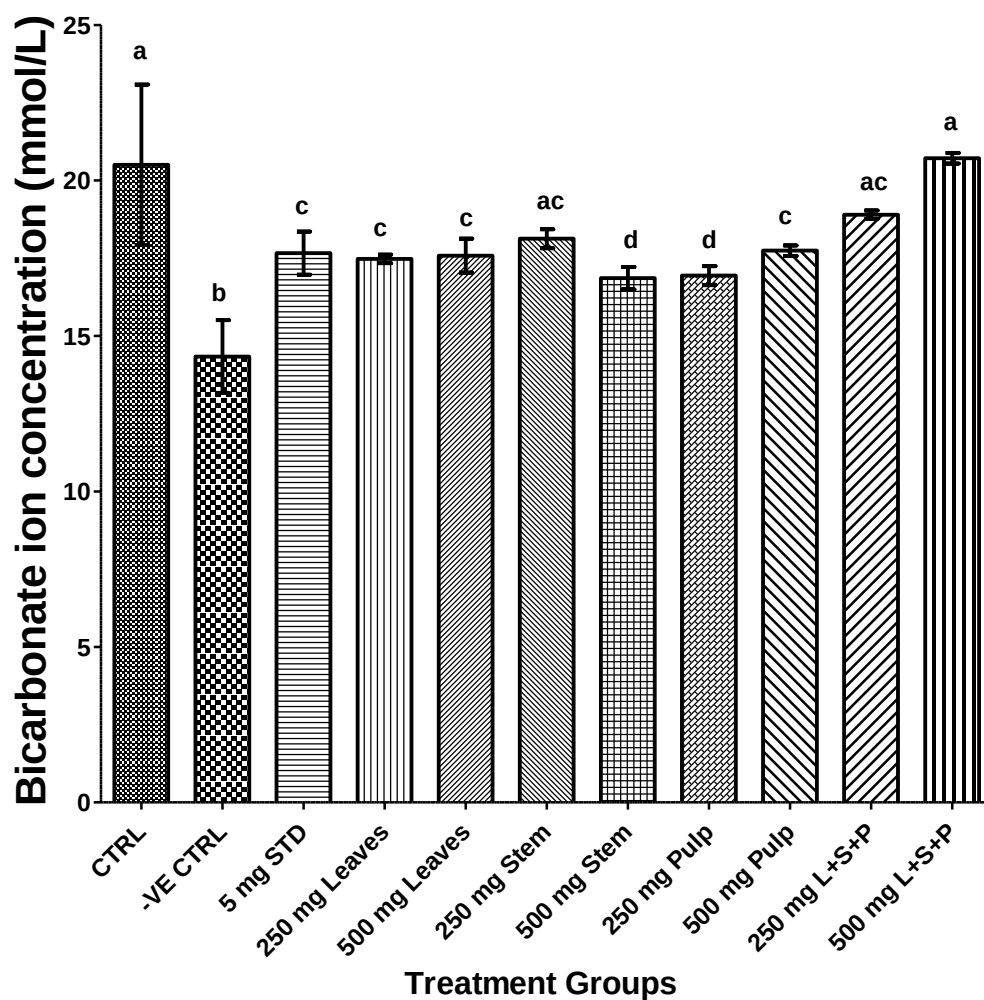


Figure. 4.23 Serum bicarbonate ion concentration (mmol/L) of rats treated with different doses of ethanolic extracts of plant parts of *A. heterophyllus*

Bars are mean $\pm$ standard deviation (n = 3). Bars bearing different alphabet letters are statistically significant ($p < 0.05$).

Chloride was significantly elevated in the negative control ($81.77 \pm 3.18\text{mmol/L}$), Control group maintained normal chloride ($57.89 \pm 2.10\text{mmol/L}$). Stem (500 mg) and Ext. Combination (500 mg) restored chloride to near-control levels (59.25 ± 2.95 and $59.45 \pm 0.89 \text{ mmol/L}$). Leaf and pulp extracts showed elevated chloride, especially at higher doses (**figure 4.24**).

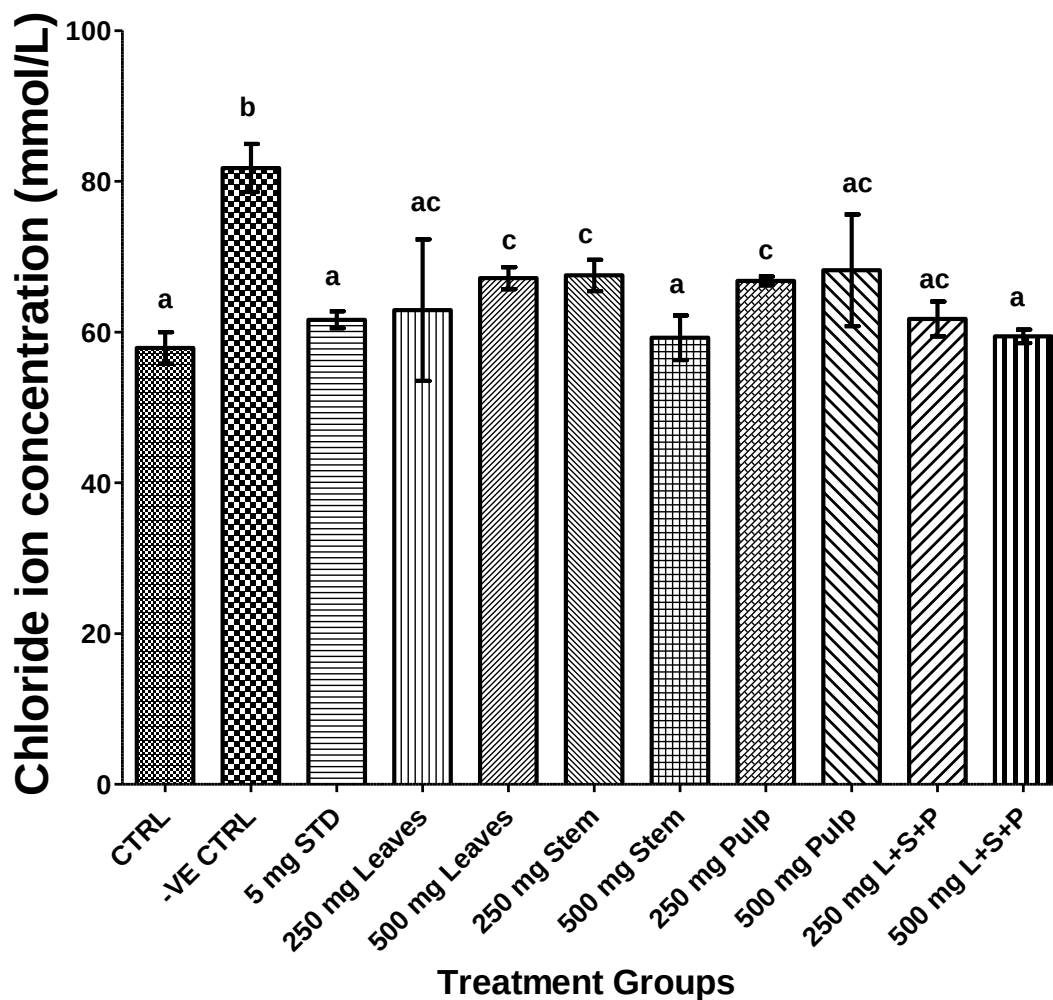


Figure. 4.24 Serum chloride ion concentration of rats treated with different doses of ethanolic extracts of plant parts of *A. heterophyllus*

Bars are mean $\pm$ standard deviation (n = 3). Bars bearing different alphabet letters are statistically significant ($p < 0.05$).

4.1.17 Effect of plant parts extract of *A. heterophyllus* on haematological parameters

White Blood Cell levels (**figure 4.25**) remained constant across most groups ($99.90 \pm 0.00 \times 10^9 \text{ \L}$), including control, negative control, and all extract treatments, Slight reductions were observed in Pulp (500 mg) ($98.33 \pm 2.71 \times 10^9 \text{ \L}$), Ext. Combination (250 mg) ($94.60 \pm 4.69 \times 10^9 \text{ \L}$), and Ext. Combination (500 mg) ($99.10 \pm 1.06 \times 10^9 \text{ \L}$).

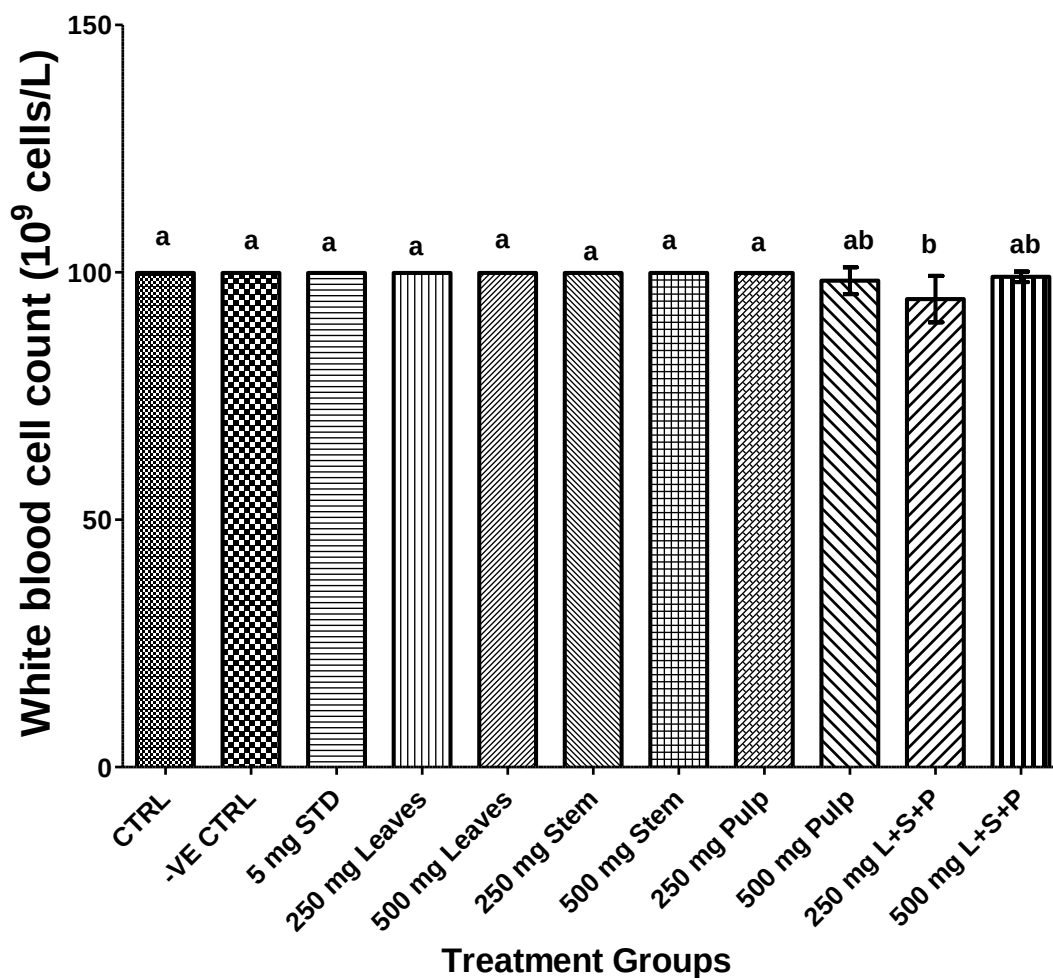


Figure. 4.25 White blood cell count (10^9 cells/L) of rats treated with different doses of ethanolic extracts of plant parts of *A. heterophyllum*

Bars are mean $\pm$ standard deviation (n = 3). Bars bearing different alphabet letters are statistically significant ($p < 0.05$).

Figure 4.26 shows Lymphocyte Count, Negative control showed a statistically significant ($p < 0.05$) elevation (54.30 ± 3.52 %), Control group maintained baseline levels (27.67 ± 0.81 %), Leaves (250 mg) showed the lowest lymphocyte count (14.50 ± 3.93 %), Stem (250 mg), Finasteride, and Pulp (250 mg) showed elevated lymphocyte counts (45.30 – 48.30 %), Ext. Combination (250 mg) and (500 mg) showed intermediate lymphocyte levels (37.97 ± 6.25 % and 44.67 ± 5.24 %).

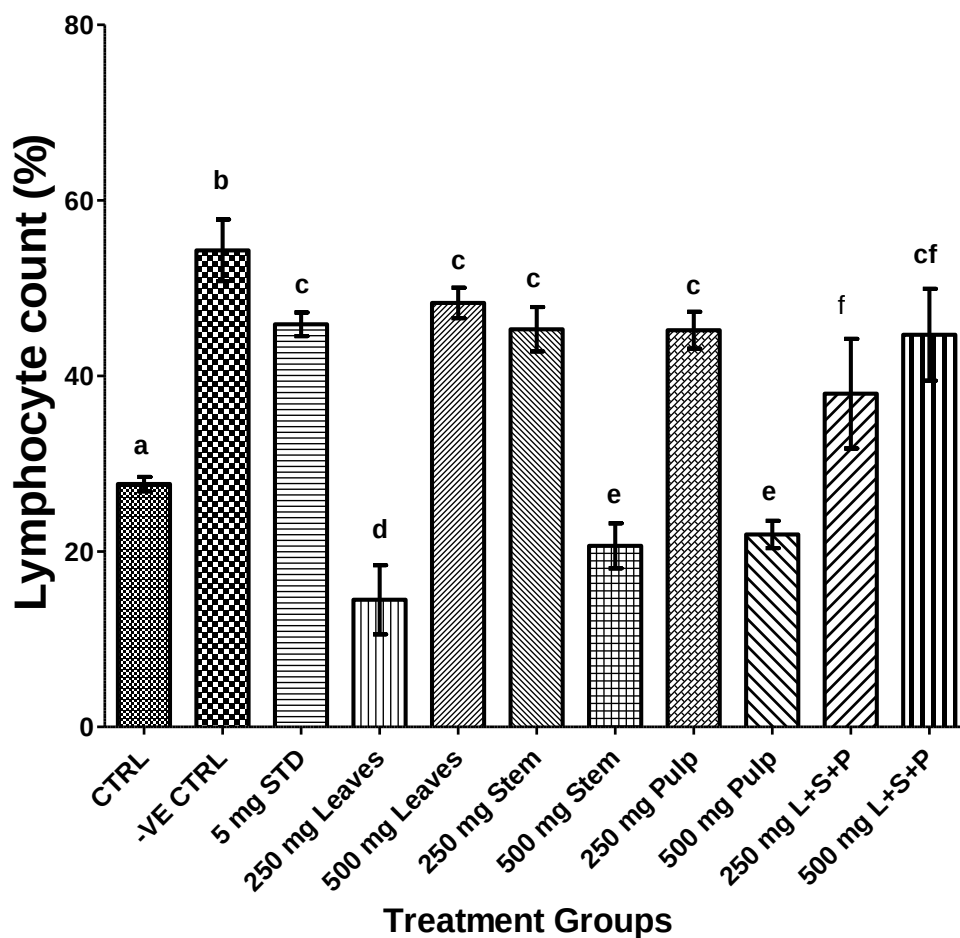


Figure. 4.26 % Lymphocyte count of rats treated with different doses of ethanolic extracts of plant parts of *A. heterophyllus*

Bars are mean $\pm$ standard deviation (n = 3). Bars bearing different alphabet letters are statistically significant ($p < 0.05$).

Control and negative control groups had baseline monocyte levels ($8.63 \pm 0.15 \%$ and $7.67 \pm 0.68 \%$) in **figure 4.27**. Finasteride, Stem (250 mg), Pulp (500 mg), and Ext. Combination (500 mg) showed significantly elevated monocyte counts (11.90–19.33 %), while Leaves (250 mg & 500 mg) and Stem (500 mg) maintained monocyte levels similar to control

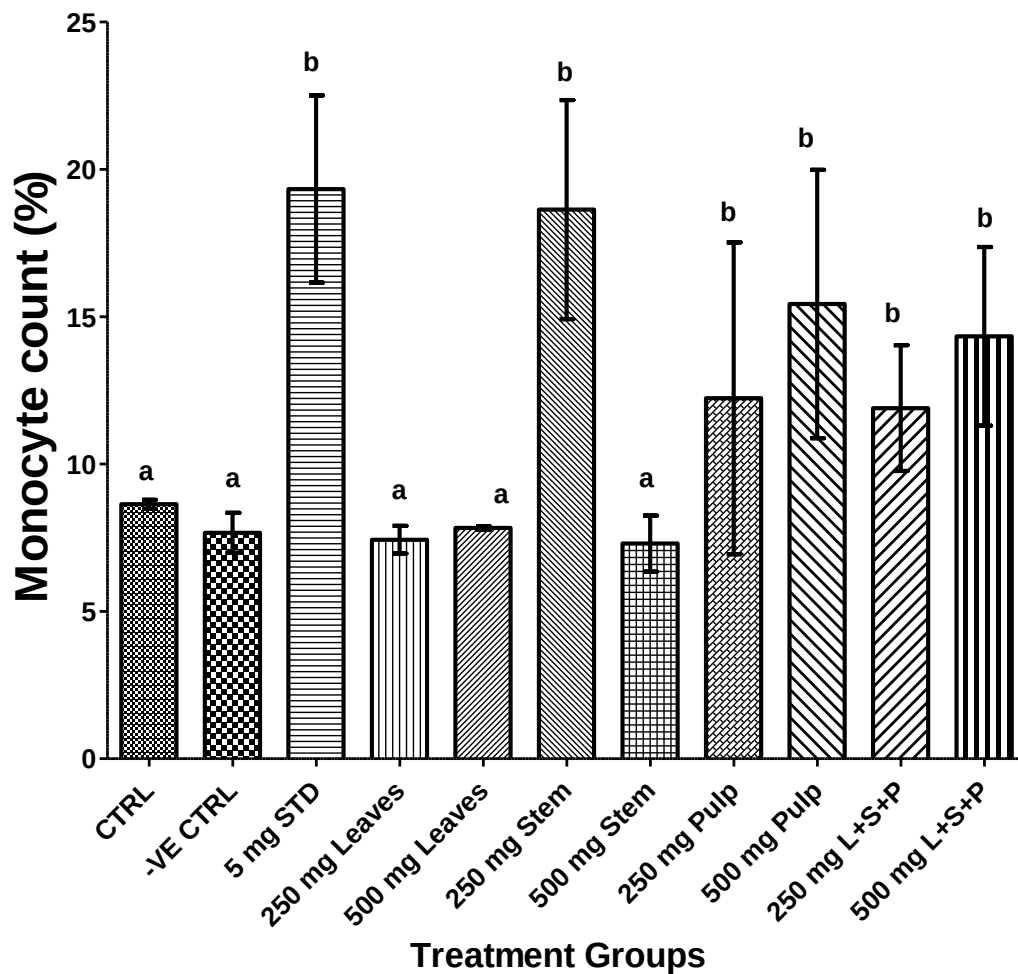


Figure. 4.27 Monocyte count (%) of Rats Treated with Different Doses of Ethanolic Extracts of Plant Parts of *A. heterophyllus*

Bars are mean $\pm$ standard deviation (n = 3). Bars bearing different alphabet letters are statistically significant (p<0.05).

. **Figure 4.28** shows the Control and negative control groups had similar HB levels (14.23 ± 1.05 g/dL and 14.10 ± 0.56 g/dL). All treatment groups showed elevated HB, with Pulp (500 mg) recording the highest value (18.30 ± 2.39 g/dL), followed closely by Stem (500 mg) and Stem (250 mg) , although no statistically significant differences were noted across treatments.

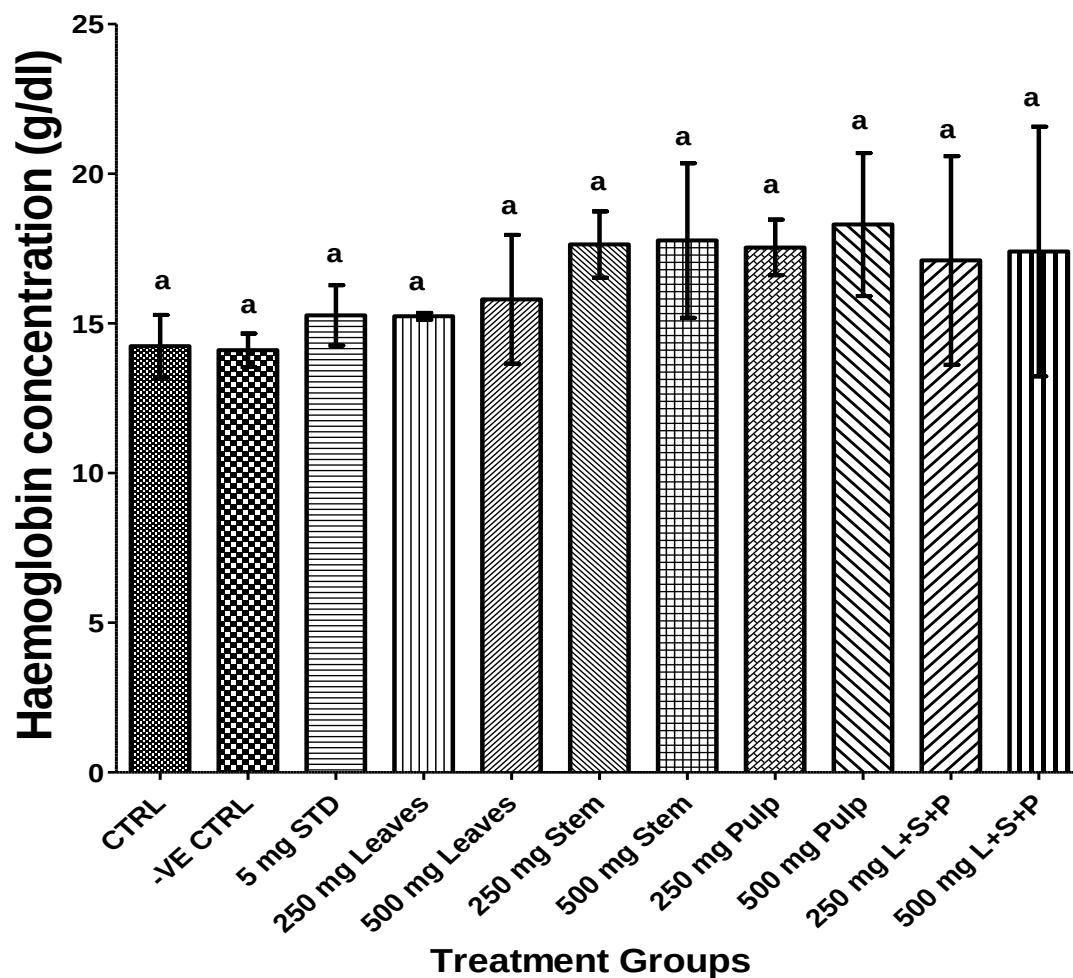


Figure. 4.28 Haemoglobin concentration (g/dL) of rats treated with different doses of ethanolic extracts of plant parts of *A. heterophyllus*

Bars are mean $\pm$ standard deviation (n = 3). Bars bearing different alphabet letters are statistically significant ($p < 0.05$).

In **figure 4.29**, Ext. Combination (500 mg) showed the highest RBC count ($14.80 \pm 3.20 \times 10^{12}/\text{dL}$), significantly different ($p < 0.05$) from Leaves (500 mg) ($11.00 \pm 2.00 \times 10^{12}/\text{dL}$). Most other treatments maintained RBC counts within the control range ($11.16\text{--}13.25 \times 10^{12}/\text{dL}$).

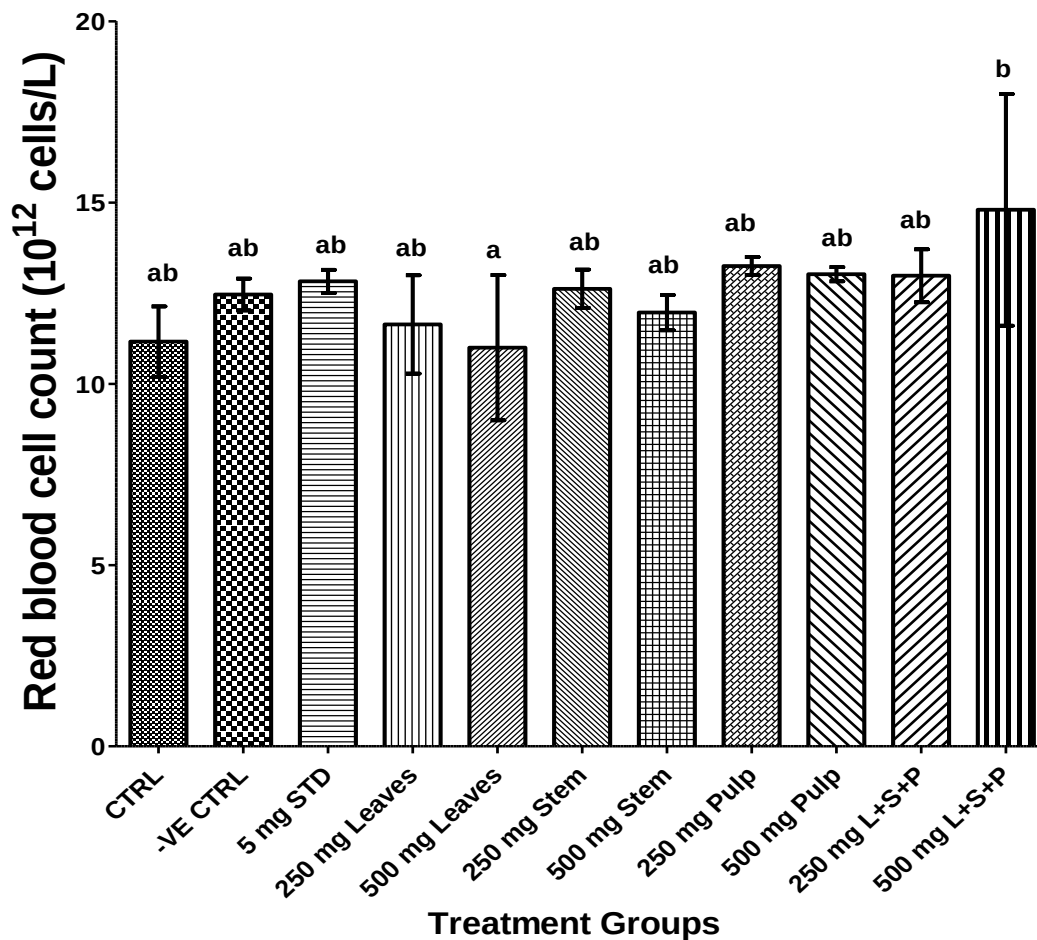


Figure. 4.29 Red blood cell count (10^{12} cells/L) of rats treated with different doses of ethanolic extracts of plant parts of *A. heterophyllus*

Bars are mean $\pm$ standard deviation ($n = 3$). Bars bearing different alphabet letters are statistically significant ($p < 0.05$).

Figure 4.30 showed that the MCV remained consistent across all groups (35.03 - 37.03 fL) with no significant differences .

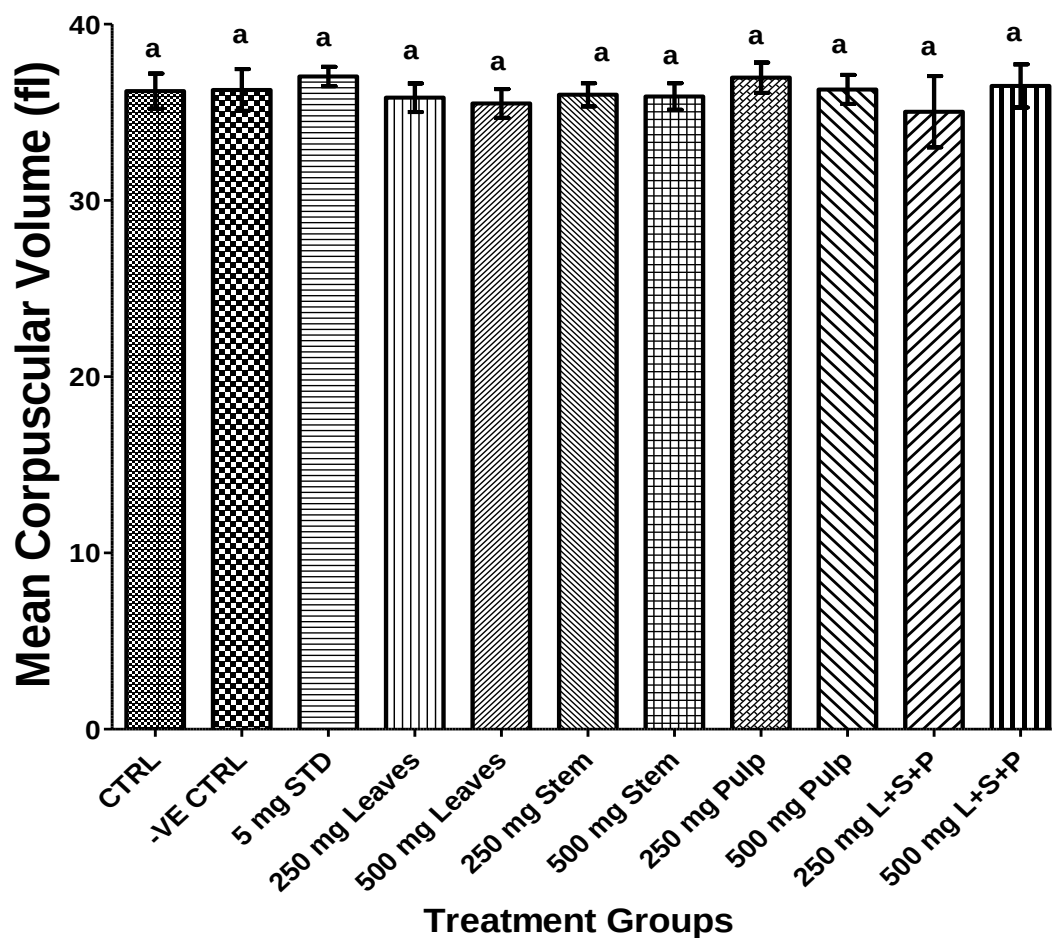


Fig. 4.30 Mean corpuscular volume (fl) of rats treated with different doses of ethanolic extracts of plant parts of *A. heterophyllus*

Bars are mean $\pm$ standard deviation (n = 3). Bars bearing different alphabet letters are statistically significant ($p < 0.05$).

Mean Corpuscular Hemoglobin (MCH) value remained almost the same across the group showing no statistically significant differences (**figure 4.31**).

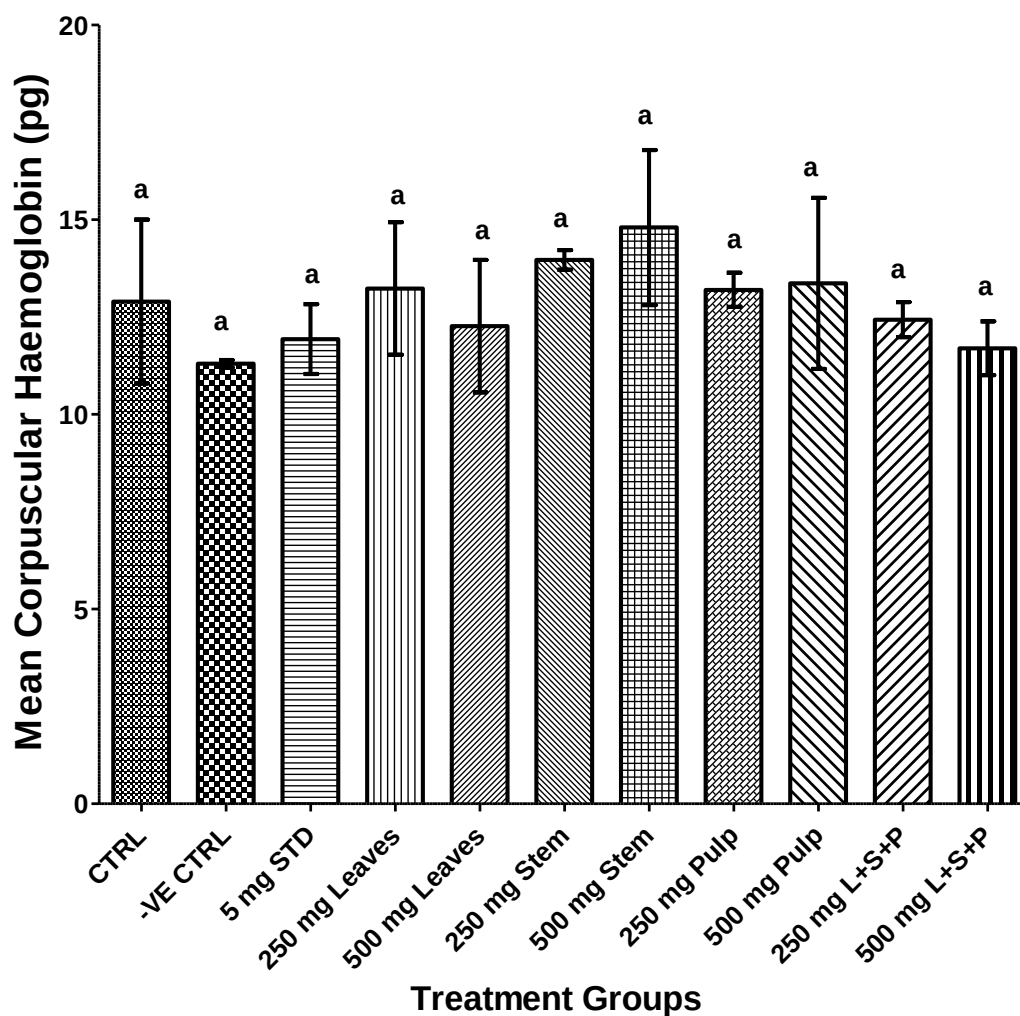


Fig. 4.31 Mean corpuscular haemoglobin (pg) of rats treated with different doses of ethanolic extracts of plant parts of *A. heterophyllus*

Bars are mean $\pm$ standard deviation (n = 3). Bars bearing different alphabet letters are statistically significant ($p < 0.05$).

Figure 4.32 shows Mean Corpuscular Hemoglobin Concentration (MCHC) was lowest in the negative control (31.30 ± 0.70 g/dL), Stem (500 mg) showed the highest MCHC (41.33 ± 4.92 g/dL), other extracts maintained MCHC within normal range with no statistical difference.

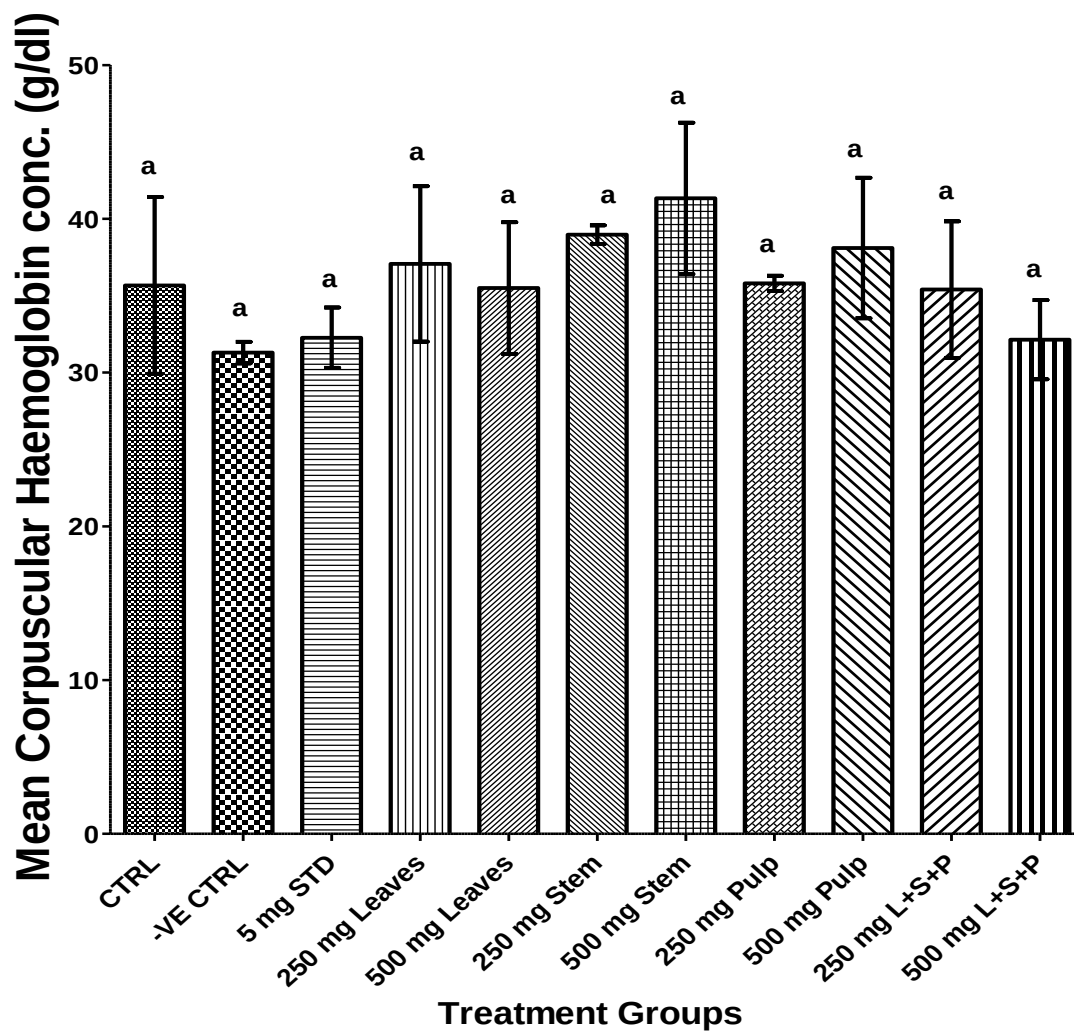


Figure. 4.32 Mean corpuscular haemoglobin concentration (g/dl) of rats treated with different doses of ethanolic extracts of plant parts of *A. heterophyllus*

Bars are mean $\pm$ standard deviation (n = 3). Bars bearing different alphabet letters are statistically significant ($p < 0.05$).

Platelet counts as observed in **figure 4.33** were stable across all groups ($8806\text{--}9933 \times 10^9/\text{L}$), with no statistically significant differences. Stem (500 mg) showed the highest PLT ($9933.00 \pm 63.02 \times 10^9/\text{L}$),

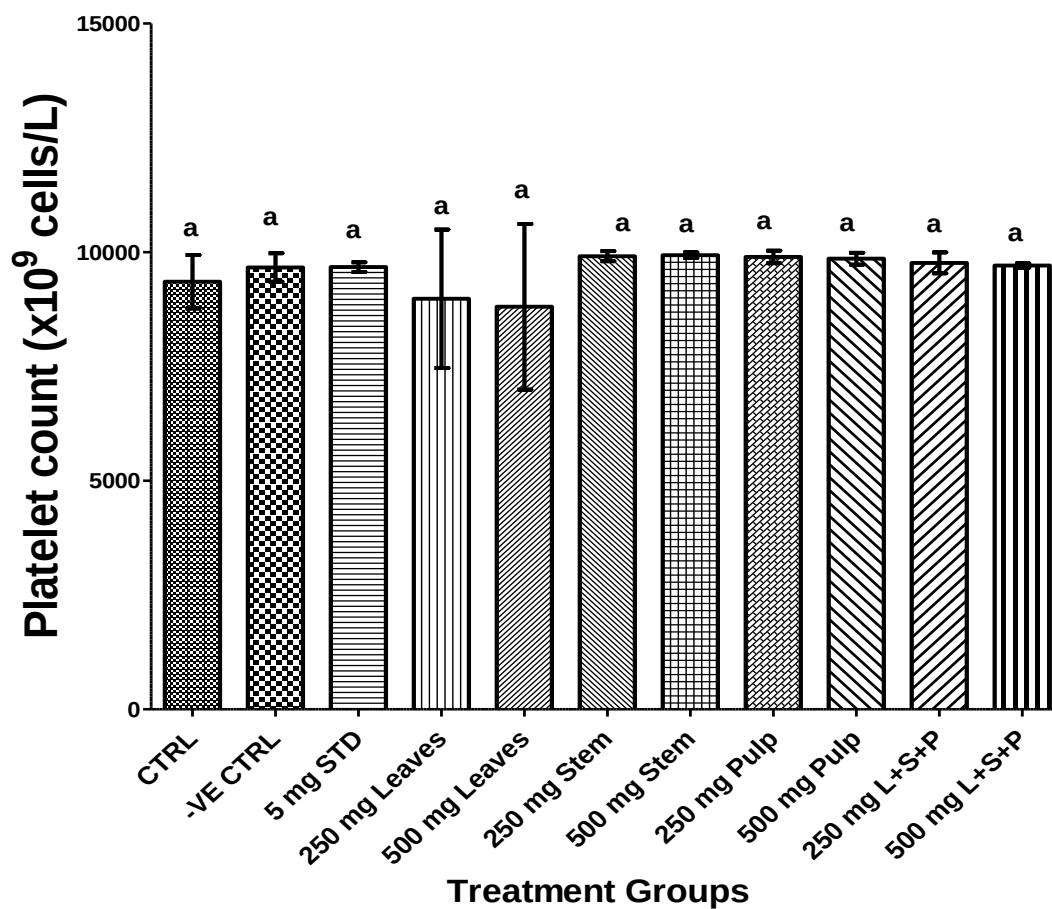


Figure. 4.33 Platelet count (x10⁹ cells/L) of rats treated with different doses of ethanolic extracts of plant parts of *A. heterophyllus*

Bars are mean $\pm$ standard deviation (n = 3). Bars bearing different alphabet letters are statistically significant (p<0.05).

4.1.17 Effect of plant parts extract of *A. heterophyllus* on organ/relative organ weight

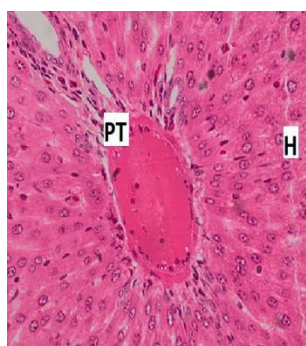
Table 4.24 Body weight and organ weight measurements

Group	Body weight (g)	Organ weights (g)			Relative Organ weights (g)		
		Liver	Kidney	Prostate	Liver	Kidney	Prostate
1	173.4 ± 15.8 [□]	6.82 ± 0.86 [□]	0.60 ± 0.10 [□]	0.41 ± 0.05 [□]	3.93 ± 0.29 [□]	0.34 ± 0.03 [□]	0.24 ± 0.05 [□]
2	196.2 ± 7.4 ^{□□}	7.34 ± 0.25 ^{□□}	0.62 ± 0.04 [□]	0.85 ± 0.21 [□]	3.74 ± 0.02 [□]	0.31 ± 0.01 [□]	0.43 ± 0.10 [□]
3	233.3 ± 23.7 [□]	9.36 ± 0.98 [□]	0.72 ± 0.03 [□]	0.71 ± 0.12 [□]	4.01 ± 0.04 [□]	0.31 ± 0.02 [□]	0.31 ± 0.07 [□]
4	205.9 ± 13.4 ^{□□}	8.06 ± 0.59 ^{□□}	0.64 ± 0.08 [□]	0.83 ± 0.17 [□]	3.91 ± 0.12 [□]	0.31 ± 0.02 [□]	0.40 ± 0.06 [□]
5	198.1 ± 22.9 ^{□□}	7.90 ± 1.02 ^{□□}	0.61 ± 0.11 [□]	0.54 ± 0.13 [□]	4.01 ± 0.08 [□]	0.31 ± 0.02 [□]	0.27 ± 0.04 [□]
6	203.4 ± 7.8 ^{□□}	7.17 ± 0.24 [□]	0.63 ± 0.01 [□]	0.82 ± 0.21 [□]	3.53 ± 0.16 [□]	0.31 ± 0.01 [□]	0.40 ± 0.10 [□]
7	167.3 ± 18.9 [□]	6.87 ± 0.44 [□]	0.56 ± 0.03 [□]	0.71 ± 0.15 ^{□□}	4.16 ± 0.69 [□]	0.34 ± 0.03 [□]	0.43 ± 0.09 [□]
8	242.5 ± 4.6 [□]	8.30 ± 0.41 [□]	0.80 ± 0.05 [□]	0.95 ± 0.07 [□]	3.42 ± 0.19 [□]	0.33 ± 0.03 [□]	0.39 ± 0.04 [□]
9	229.4 ± 26.1 [□]	7.20 ± 0.73 [□]	0.76 ± 0.04 [□]	0.94 ± 0.13 [□]	3.14 ± 0.07 [□]	0.34 ± 0.04 [□]	0.41 ± 0.03 [□]
10	228.8 ± 24.6 [□]	6.66 ± 0.62 [□]	0.71 ± 0.01 ^{□□}	0.83 ± 0.18 [□]	2.94 ± 0.50 [□]	0.31 ± 0.03 [□]	0.36 ± 0.06 ^{□□}
11	173.8 ± 25.6 [□]	5.82 ± 0.88 [□]	0.56 ± 0.08 [□]	0.74 ± 0.19 ^{□□}	3.35 ± 0.05 [□]	0.32 ± 0.00 [□]	0.43 ± 0.14 [□]

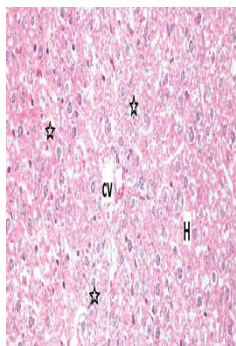
Values are expressed as mean ± SD (n = 3). Different superscripts (□, □, □) indicate statistical significance at p < 0.05 within the same column. Groups sharing the same letter are not significantly different.

4.1.18 Histopathological Examination of Liver, Kidneys and prostate of Rats treated with plant parts Extracts of *A. heterophyllus*

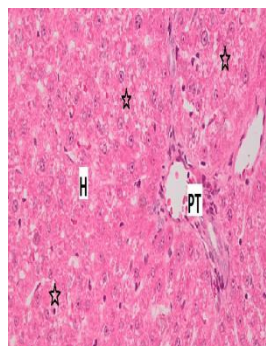
4.18.1 Liver histology of rats treated with ethanol plant parts extracts of *A. Heterophyllus*



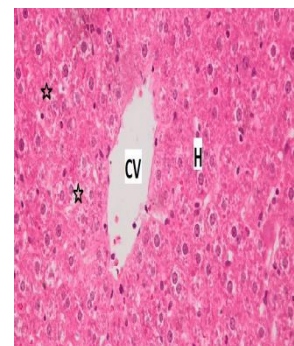
GROUP 1(A)



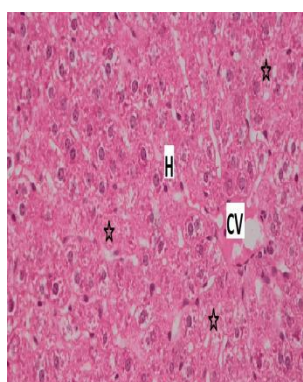
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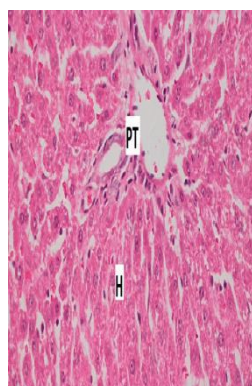
GROUP 3(C)



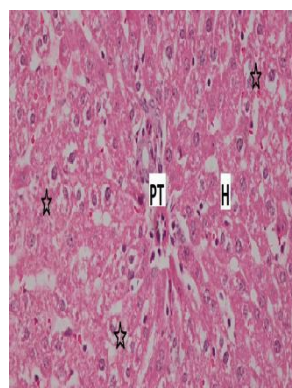
GROUP 4 (D)



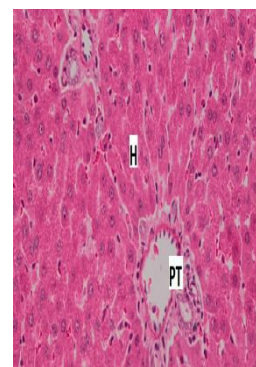
GROUP 5 (E)
GROUP 8 (H)

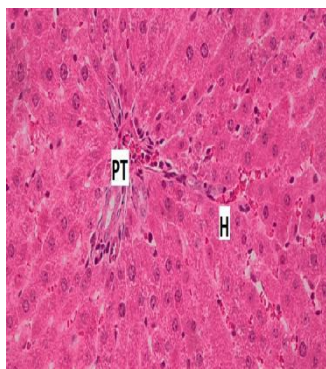


GROUP 6 (F)

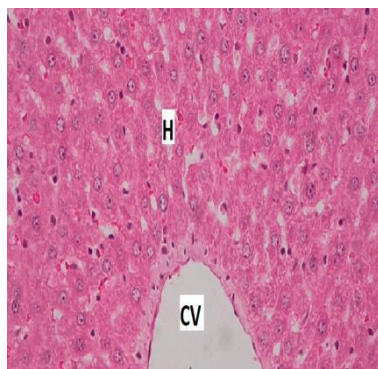


GROUP 7 (G)

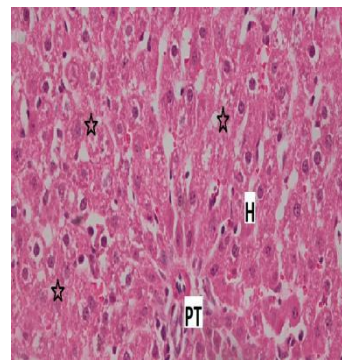




GROUP 9 (I)



GROUP 10 (J)



GROUP 11 (K)

A - Plate 4.3. Group 1: Photomicrograph of a rat liver in the control group showing normal hepatocytes (H) and portal triad (PT). No pathological changes were observed (x400). Stain: hematoxylin and eosin (H and E).

B - Plate 4.4: Group 2: Photomicrograph of liver of TP induced BPH untreated rats showing hepatocytes (H) and and central vein (CV) undergoing severe degeneration and necrosis (star) x400 , stain:H and E.

C- Plate 4.5: Group 3: Photomicrograph of the liver of rats treated with Finasteride (5mg/ kg b.w) for 21 days showing hepatocytes (H) and portal triad (PT) undergoing mild degeneration and necrosis (star) x400. Stain: H&E.

D -Plate 4.6: Group 4: Photomicrograph of the liver of rat treated with 250mg/kg B.W of ethanol stem bark extract of *A. heterophyllus* for 21 days showing hepatocytes (H) undergoing mild degeneration and necrosis (star) and an enlarged central vein (CV). x 400, stain:H and E

E - Plate 4.7: Group 5: Photomicrograph of the liver of rat treated with 500 mg/kg B.W of ethanol stem bark extract of *A. heterophyllus* for 21 days showing hepatocytes (H) undergoing mild degeneration and necrosis (star) and central vein (CV). x400, stain : H and E

F- Plate 4.8. Group 6: Photomicrograph of the liver of rat treated with 250 mg/kg B.W of ethanol leaves extract of *A. heterophyllus* for 21 days showing normal hepatocytes (H) and portal triad (PT). x 400, stain H and E .

G - Plate 4.9: Group 7: Photomicrograph of the liver of rat treated with 500 mg/kg B.W of ethanol leaves extract of *A. heterophyllus* for 21 days showing hepatocytes (H) and portal triad (PT) undergoing severe degeneration and necrosis (star) .x400, stain : H and E.

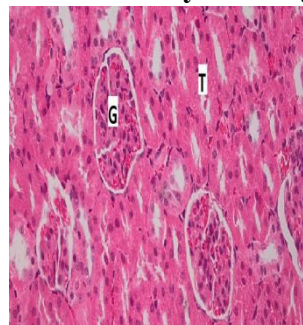
H -Plate 4.10: Group 8: Photomicrograph of the liver of rat treated with 250 mg/kg B.W of ethanol pulp extract of *A. heterophyllus* for 21 days showing normal hepatocytes (H) and portal triad (PT). x 400, stain:H and E.

I - Plate 4.11: Group 9: Photomicrograph of the liver of rat treated with 500 mg/kg B.W of ethanol pulp extract of *A. heterophyllus* for 21 days showing normal hepatocytes (H) and portal triad (PT). x 400, Stain: H and E

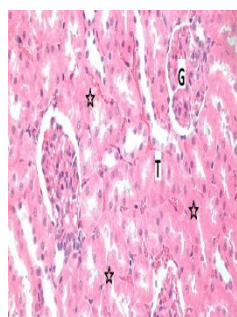
J - Plate 4.12: Group 10 : Photomicrograph of the liver of rat treated with 250 mg/kg B.W of ethanol stem bark + leaves+ pulp extracts of *A. heterophyllus* for 21 days showing normal hepatocytes (H) and central vein (CV). x 400 , Stain : H and E

K - Plate 4.13: Group 11 : photomicrograph of the liver of rat treated with 250 mg/kg B.W of ethanol stem bark + leaves+ pulp extracts of *A. heterophyllus* for 21 days showing hepatocytes (H) and portal Triad (PT) undergoing moderate degeneration and necrosis (star). x400, stain H and E

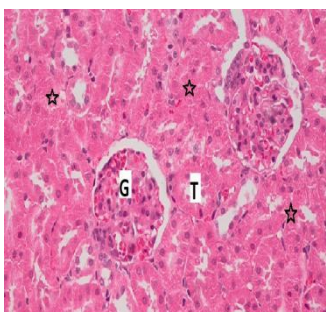
4.18.2 Kidney histology of rats treated with ethanol plant parts extracts of *A. Heterophyllus*



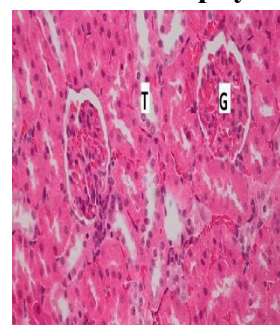
GROUP 1 (A)



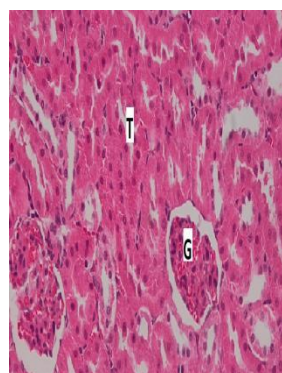
GROUP 2 (B)



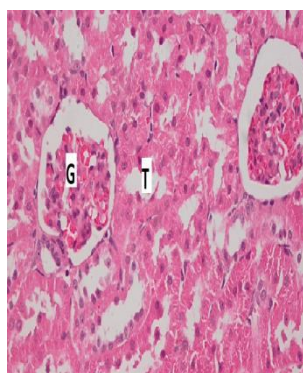
GROUP 3 (C)



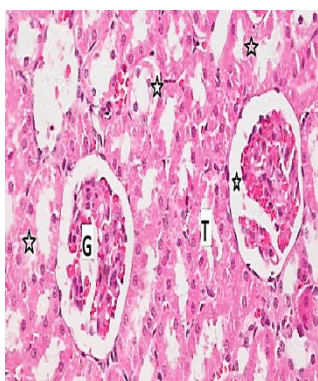
GROUP 4 (D)



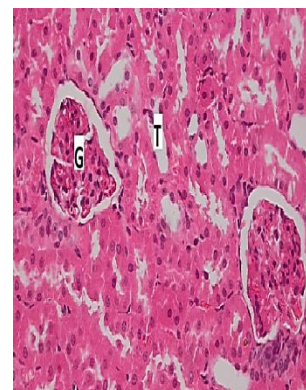
GROUP 5 (E)



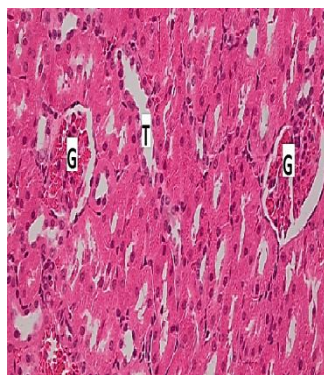
GROUP 6 (F)



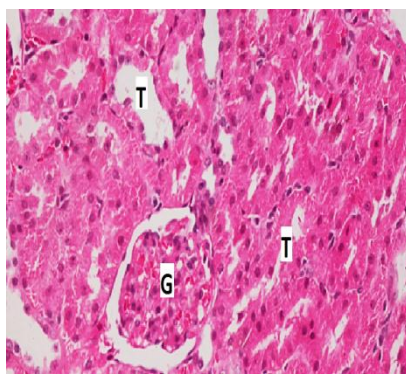
GROUP 7 (G)



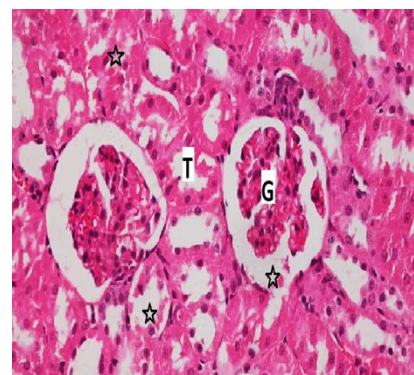
GROUP 8 (H)



GROUP 9 (I)



GROUP 10 (J)



GROUP 11

A - Plate 4.14: Group 1: Photomicrograph of rat kidney in the control group showing normal glomerulus (G) and renal tubules (t). No pathological changes observed x 400, Stain: H and E.

B - Plate 4.15: Group 2 kidney: Photomicrograph of the kidney of a TP induced untreated BPH showing the glomerulus (G) and severe degeneration and necrosis of the tubular epithelial cells (star). x 400 , Stain : H and E

C - Plate 4.16: group 3: Photomicrograph of the kidney of a rat treated with Finasteride (5mg/Kg B.W) for 21 days showing the glomerulus(G) and mild Degeneration and Necrosis of the tubular epithelial cells (star) x400, Stain: H and E

D - Plate 4.17: Group 4: Photomicrograph of the kidney of a rat treated with 250mg/kg B.W of ethanol extract of stem bark of *A. heterophyllus* for 21 days showing normal glomerulus (G) and renal tubules (t). x 400, stain : H and E

E - Plate 4.18: Group 5 kidney: Photomicrograph of the kidney of a rat treated with 500mg/kg B.W of ethanol extract of stem bark of *A. heterophyllus* for 21 days showing normal glomerulus (G) and renal tubules (t). x400, stain : H and E. .

F - Plate 4.19: Group 6: Photomicrograph of the kidney of a BPH induced rat treated with 250mg/kg B.W of ethanol extract of leaves of *A. heterophyllus* for 21 days showing normal glomerulus (G) and renal tubules (t). x 400 , stain : H and E..

G - Plate 4.20: Group 7 : Photomicrograph of the kidney of a BPH induced rat treated with 500mg/kg B.W of ethanol extract of leaves of *A. heterophyllus* for 21 days showing the glomerulus (G) and severe degeneration and necrosis of the tubular epithelial cells (star). x400 , stain : H and E.

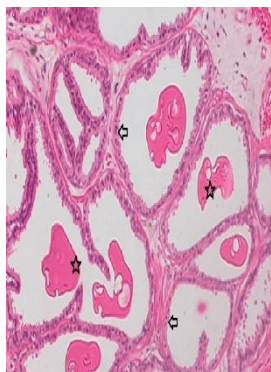
H - Plate 4.21: Group 8: Photomicrograph of the kidney of a BPH induced rat treated with 250 mg/kg B.W of ethanol extract of pulp of *A. heterophyllus* for 21 days showing normal glomerulus (G) and renal tubules (t). x 400, stain : H and E.

I - Plate 4.22: Group 9 kidney: Photomicrograph of the kidney of a rat treated with 500mg/kg B.W of ethanol extract of pulp of *A. heterophyllus* for 21 days showing normal glomerulus (G) and renal tubules (t) with no pathological changes.. x400 , stain H & E

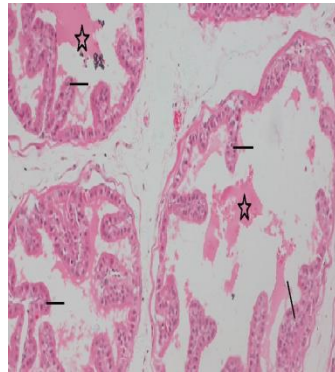
J - Plate 4.23: Group 10 kidney: photomicrograph of the kidney of a BPH induced rat treated with 250 mg/kg B.W of ethanol combination extract of stem bark + leaves + pulp of *A. heterophyllus* for 21 days showing normal glomerulus (G) and renal tubules (t). x400 , stain : H and E

K - Plate 4.24: Group 11 kidney: photomicrograph of the kidney of a BPH induced rat treated with 500mg/kg B.W of combination ethanol extract of stem bark + leaves + pulp of *A. heterophyllus* for 21 days showing the glomerulus (G) and moderate degeneration and necrosis of the tubular epithelial cells (star). x400 , stain H and E.

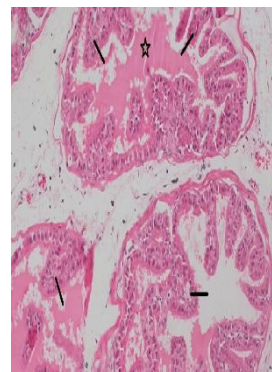
4.1.18.3 Prostate histology of rats treated with ethanol plant parts extracts of *A. Heterophyllus*



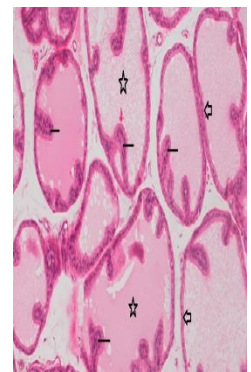
GROUP 1 (A)



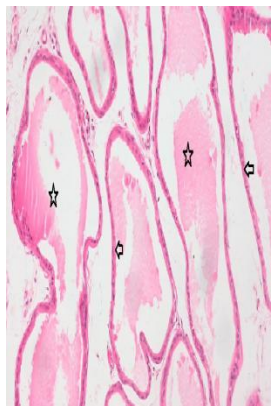
GROUP 2 (B)



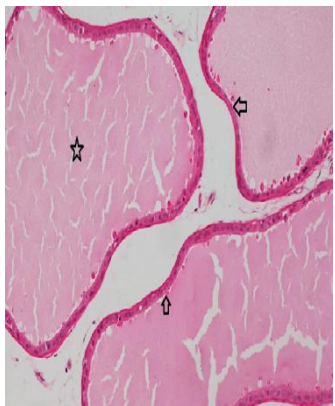
GROUP 3 (C)



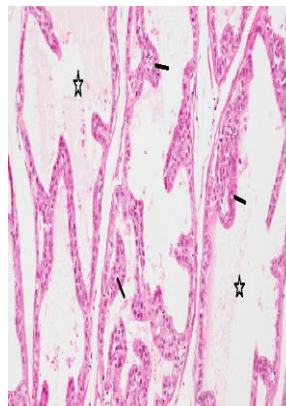
GROUP 4 (D)



GROUP 5 (E)



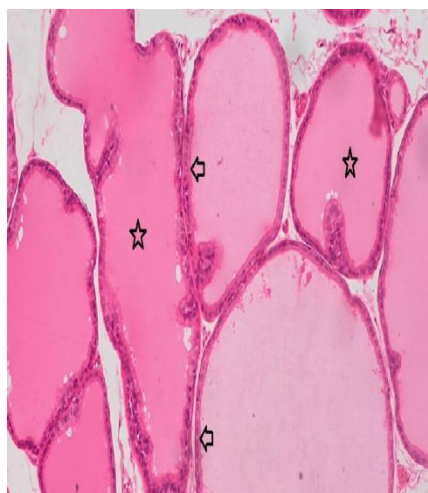
GROUP 6 (F)



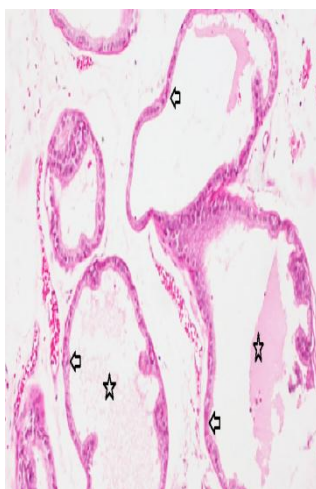
GROUP 7 (G)



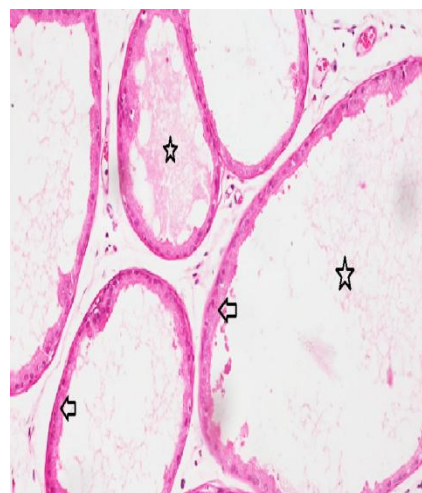
GROUP 8 (H)



GROUP 9 (I)



GROUP 10 (J)



GROUP 11 (K)

A - Plate 4. 25: Group 1: photomicrograph of the prostate gland of a rat in the control group showing normal histological architecture of the acini with pinkish secretions in the lumen (star) and normal epithelium of the gland (arrow). X400, stain: H and E

B - Plate 4.26: Group 2: Photomicrograph of the prostate gland of a TP induced BPH, untreated rat showing the acini with pinkish secretions in the lumen (star) and thick (hypertrophied) epithelium of the gland with projections of the epithelium into the lumen of the acini (lines). X400 , stain : H and E

C - Plate 4.27: Group 3: Photomicrograph of the prostate gland of BPH induced rat treated with Finasteride (5mg/kg B.W) for 21 days showing the acini with pinkish secretions in the lumen (star) and thick (hypertrophied) epithelium of the gland with many projections of the epithelium into the lumen of the acini (lines). X400, stain : H and

D - Plate 4. 28: Group 4 : Photomicrograph of the prostate gland of a BPH induced rat treated with 250mg/kg B.W of ethanol stem bark extract of *A. heterophyllus* for 21 days showing the acini with pinkish secretions in the lumen (star) and many projections of the epithelium into the lumen of the acini (lines) indicating hypertrophy. X400 , stain : H and E.

E - Plate 4.29: Group 5 : Photomicrograph of the prostate gland of a BPH induced rat treated with 500 mg/kg B.W of ethanol stem bark extract of *A. heterophyllus* for 21 days showing normal histological architecture of the acini with pinkish secretions in the lumen (star) and normal epithelium of the gland (arrow). X400 . stain: H and E .

F - Plate 4.30: Group 6 : Photomicrograph of the prostate gland of a BPH induced rat treated with 250mg/kg B.W of ethanol leaves extract of *A. heterophyllus* for 21 days showing normal histological architecture of the acini with pinkish secretions in the lumen (star) and normal epithelium of the gland (arrow). X400 , stain : H and E

G - Plate 4.31: Group 7: photomicrograph of the prostate gland of a BPH induced rat treated with 500 mg/kg B.W of ethanol leaves extract of *A. heterophyllus* for 21 days showing the acini with pinkish secretions in the lumen (star) and thick (hypertrophied) epithelium of the gland with a few projections of the epithelium into the lumen of the acini (lines). X400 , stain : H and E

H - Plate 4.32: Group 8 : photomicrograph of the prostate gland of a BPH induced rat treated with 250mg/kg B.W of ethanol pulp extract of *A. heterophyllus* for 21 days, showing the acini with pinkish secretions in the lumen (star) and thick (hypertrophied) epithelium of the gland with a few projections of the epithelium into the lumen of the acini (lines). X400 , Stain: H and E.

I - Plate 4. 33: Group 9 : photomicrograph of the prostate gland of a BPH induced rat treated with 500mg/kg B.W of ethanol pulp extract of *A. heterophyllus* for 21 days showing normal histological architecture of the acini with pinkish secretions in the lumen (star) and normal epithelium of the gland (arrow). X400 , Stain : H and

J - Plate 34: Group 10 : photomicrograph of the prostate gland of a BPH induced rat treated with 250mg/kg B.W of ethanol stem bark + leaves + pulp extract of *A. heterophyllus* for 21 days showing normal histological architecture of the acini with pinkish secretions in the lumen (star) and normal epithelium of the gland (arrow). X400 , stain: H and E.

K - Plate 4. 35: Group 11 : photomicrograph of the prostate gland of a BPH induced rat treated with 500mg/kg B.W of ethanol stem bark + leaves + pulp extract of *A. heterophyllus* for 21 days showing normal histological architecture of the acini with pinkish secretions in the lumen (star) and normal epithelium of the gland (arrow). X400 , stain : H and E.

4.2 Discussion

Recent advancements in medicinal plant research have highlighted the significant therapeutic potentials of various plant species. Studies have demonstrated that plants possess a wide array of bioactive compounds contributing to their medicinal properties. The application of techniques such as Gas Chromatography-Mass Spectrometry (GC-MS) and molecular docking studies has facilitated the identification and characterization of active compounds, paving the way for the development of novel therapeutic agents.

Gas Chromatography–Mass Spectrometry (GC–MS) is a powerful analytical technique that combines the high-resolution separation ability of gas chromatography with the structural elucidation capacity of mass spectrometry. It is particularly effective in identifying volatile and semi-volatile constituents of plant extracts, essential oils, and other complex mixtures (Jahan et al., 2020). Its sensitivity, reproducibility, and ability to generate qualitative and quantitative information make it invaluable for phytochemical screening, food chemistry, forensic science, and drug discovery (Kareem et al., 2024). In phytomedicine, GC–MS has become a key tool for linking phytochemicals to their pharmacological

activities, such as antimicrobial, antioxidant, anticancer, and pesticidal effects (Scientific Reports, 2023).

The GC-MS analyses of the stem bark, pulp, and leaf extracts of *Artocarpus heterophyllus* plant revealed a diverse array of bioactive compounds with varying pharmacological properties, underscoring the plant's therapeutic potential. The stem bark extract contained sixty four (64) compounds, predominantly fatty acids, esters, and alkanes, while the pulp extract presented a more complex profile of ninety four (94) compounds, and the leaf extract revealed forty eight (48) compounds. This variation in the number and types of compounds may be attributed to tissue-specific metabolic pathways, which influence the biosynthesis and accumulation of secondary metabolites (Pandey et al., 2017).

In the stem bark, notable constituents such as n-hexadecanoic acid, 9-octadecenoic acid, and oleic acid were identified, compounds widely recognised for their anti-inflammatory, antioxidant, and antimicrobial activities (Aparna et al., 2012; Sales-Campos et al., 2013; Alabi et al., 2018). Similarly, the pulp extract contained several bioactive fatty acids, including cis-vaccenic acid, tetradecanoic acid, and dodecanoic acid, alongside phytosterols such as squalene. These metabolites have been previously associated with antibacterial, hypolipidemic, anticancer, and immunostimulatory effects (Hamazaki et al., 2016; Zih-Rou et al., 2009; Renugadevi et al., 2021). In contrast, the leaf extract displayed a relatively smaller number of compounds but retained key bioactive molecules such as tetradecane, phenolic derivatives, and hexadecane, which have been reported to have antimicrobial, cytotoxic, and antioxidant properties (Banakar & Jayaraj, 2018; Ferdosi et al., 2022).

The comparative profile indicates that the pulp extract is richer in both the number and diversity of compounds compared to the stem bark and leaves, which may suggest its greater pharmacological versatility. For instance, unique compounds such as succinic acid derivatives and pyrrolidine

derivatives were identified in the pulp extract, both of which have been associated with antidiabetic and antifungal activities (Adewole et al., 2018). The presence of such compounds could enhance the therapeutic potential of the pulp fraction in comparison to other plant parts. However, the stem bark and leaves remain important due to the abundance of common fatty acids with well-established pharmacological activities, particularly anti-inflammatory and antimicrobial actions.

A recurring trend across all three plant parts is the prevalence of fatty acids and hydrocarbons, which are typical of many medicinal plants and contribute significantly to antimicrobial and antioxidant mechanisms (Ajayi & Akinsanya, 2023). For example, hexadecane and its derivatives were consistently present across all extracts and have been documented to exhibit antibacterial and antifungal properties (Ferdosi et al., 2022). Likewise, phenolic and ester compounds identified across the samples contribute to the overall antioxidant defence, which is vital in mitigating oxidative stress-related diseases (Milaeva et al., 2010).

In summary, while the stem bark and leaf extracts contain several bioactive metabolites of pharmacological importance, the pulp extract stands out due to its higher chemical diversity and unique compounds. This suggests that the pulp may represent a particularly promising source of bioactive agents for drug discovery and development. Nonetheless, the overlap in fatty acid and hydrocarbon constituents across all extracts indicates a shared pharmacological basis, particularly in antimicrobial and antioxidant activities, reinforcing the plant's medicinal value.

Fourier Transform Infrared (FTIR) spectroscopy is a rapid, non-destructive analytical method that identifies functional groups present in plant extracts by analysing vibrational frequencies of chemical bonds (Parastar et al., 2020). This technique provides valuable insights into the chemical profile of plant tissues, especially in relation to their bioactive potential, since the presence of certain functional groups such as hydroxyl, carbonyl, amine, or aromatic bonds often correlates with antimicrobial,

antioxidant, or pharmacological activities (Kumar et al., 2018; Sahoo et al., 2021). The FTIR spectra of the leaf, stem bark, and pulp extracts of the studied plant reveal both overlapping and unique features, reflecting the chemical diversity distributed across different plant parts. The FTIR comparison highlights both shared and unique functional features across the three plant parts. All extracts contained hydroxyl groups, pointing to the ubiquity of alcohols and phenolic compounds in the species. However, the leaf extract was more chemically diverse, containing sulfur- and nitro-related functionalities; the stem bark extract was distinct in its nitrogen-enriched profile, including isothiocyanates, isocyanates, and amines; while the pulp extract exhibited stronger hydrocarbon and aromatic signatures.

The leaf extract was characterised by broad and sharp peaks corresponding to hydroxyl (O–H) groups, both in free alcohols and in carboxylic acids, as well as distinct C–H stretching bands for alkanes and alkenes. Additional features such as sulfonate (S=O), nitro compounds (N–O), and isothiocyanate (N=C=S) vibrations suggest the presence of sulfur- and nitrogen-containing metabolites, which are often associated with antimicrobial and pesticidal properties (Akinmoladun et al., 2020). The detection of carbon dioxide (O=C=O) further points to the presence of carbonate-related structures or absorbed atmospheric CO₂. Collectively, the leaf spectrum indicates a chemically diverse profile, rich in hydroxylated, unsaturated, and heteroatom-containing compounds that may contribute to its medicinal value.

In contrast, the stem bark extract displayed a broader range of nitrogen-related functional groups, including isocyanates (N=C=O), isothiocyanates (N=C=S), ammonium (N–H), and primary amines. Peaks corresponding to C–N and C–F stretching also appeared, which are less common in plant extracts but may reflect specialised secondary metabolites accumulated in bark tissues as defence mechanisms. The presence of alkene and β -lactam carbonyl stretches at $\sim 1618\text{ cm}^{-1}$ is significant,

since β -lactams are associated with antimicrobial properties. Compared to the leaf extract, the stem bark shows a more nitrogen-rich profile, suggesting its role as a reservoir of protective compounds, consistent with its biological role as a barrier against environmental stressors.

The pulp extract, on the other hand, was dominated by hydrocarbon and aromatic functionalities. Strong C–H stretches from alkanes and aldehydes were evident, along with C=C stretching of alkenes and aromatic C–H out-of-plane bending. The detection of aromatic amine or ester bonds indicates the presence of phenolic derivatives or esterified compounds, which are typically associated with antioxidant and antimicrobial activity (Nandiyanto et al., 2019). While the pulp shared O–H bands with the leaf and bark, its weaker intensity suggests a relatively lower abundance of hydroxyl-rich compounds. Instead, its spectral profile emphasises saturated and aromatic frameworks, aligning with the pulp's possible role in metabolite storage and nutritional functions. These findings indicate a compartmentalised distribution of bioactive compounds across plant tissues, which could have important implications for their pharmacological applications.

Amino acid profiling provided complementary biochemical evidence. The stem bark extract was particularly rich in proline (42.16 ppm), an amino acid crucial in wound healing and stress tolerance (Szabados & Savouré, 2010). The leaf extract contained high levels of threonine (16.39 ppm), isoleucine (10.06 ppm), and arginine (9.16 ppm), all of which are essential in protein synthesis, immune response, and cardiovascular health. The pulp extract was unique in its high concentration of tyrosine (29.70 ppm) and tryptophan (3.61 ppm). Tyrosine serves as a precursor for neurotransmitters such as dopamine, while tryptophan is a precursor of serotonin and melatonin, both essential for mood regulation and sleep (Fernstrom, 2013). The detection of methionine, cystine, and glutamate further strengthens the nutritional potential of the extracts, as these amino acids are vital in methylation reactions, antioxidant defence (via glutathione synthesis), and neurotransmission.

When integrated, the FTIR, GC–MS, and amino acid data point toward a synergistic phytochemical–nutritional profile. Functional groups identified via FTIR (hydroxyl, carbonyl, amine, and alkene moieties) corresponded well with the compounds identified in GC–MS, such as fatty acid esters, terpenoids, and phenolic derivatives. Meanwhile, the amino acid richness complements these secondary metabolites by contributing essential nutritional and physiological benefits. The stem bark emerges as the most chemically diverse part, containing both high phytochemical complexity and the highest amino acid concentration (notably proline). The leaf extract is enriched in immune-boosting and metabolic amino acids (threonine, isoleucine, arginine), aligning with its phytochemical antioxidant profile. The pulp extract, while less diverse in secondary metabolites, is a reservoir of neurotransmitter precursors (tyrosine, tryptophan), suggesting potential neuromodulatory benefits.

The fatty acid composition of the stem bark, leaf, and pulp extracts revealed both qualitative and quantitative differences, reflecting their unique biochemical roles and potential health benefits. Across all three extracts, oleic acid (C18:1c) was predominant, particularly in the stem bark (57.15%), compared to the leaf (20.23%) and pulp (21.77%). Oleic acid, a monounsaturated fatty acid (MUFA), is well recognised for its cardioprotective, anti-inflammatory, and cholesterol-lowering effects, making the stem bark extract particularly valuable as a nutraceutical source (Sales-Campos *et al.*, 2013). Linoleic acid (C18:2c), an essential polyunsaturated fatty acid (PUFA), was highest in the leaf (7.81%) followed by stem bark (6.67%) and pulp (3.72%). As a precursor for arachidonic acid and eicosanoids, linoleic acid supports immune function, growth, and skin integrity (Galli & Calder, 2009). Similarly, α -linolenic acid (C18:3), an omega-3 fatty acid with neuroprotective and anti-inflammatory roles, was relatively higher in the stem bark (5.70%) compared to the leaf (4.77%) and pulp (2.83%), suggesting the stem bark as a stronger source of omega-3 precursors (Simopoulos, 2016).

Interestingly, stearic acid (C18:0) was significantly abundant in the pulp (17.11%), compared to the leaf (5.18%) and stem bark (4.64%). Although stearic acid is a saturated fatty acid (SFA), it is considered neutral regarding plasma cholesterol and may contribute to structural roles in plant tissues (Hunter et al., 2010). In contrast, the pulp also exhibited a high concentration of trans-oleic acid (C18:1t, 17.09%), which, alongside the leaf extract (15.82%), suggests possible isomerisation or endogenous biosynthesis. While industrial trans fats are associated with cardiovascular risks, natural trans fatty acids from plant sources may display less harmful or even beneficial roles (Brouwer *et al.*, 2010). The leaf extract stood out for its higher proportions of elaidic acid (C18:2t, 17.99%), lauric acid (C12, 8.74%), and behenic acid (C22, 2.90%), compared to the other extracts. Lauric acid has documented antimicrobial and antiviral activities, commonly reported in coconut oil (Dayrit, 2015). Behenic acid, a long-chain fatty acid, is important in membrane fluidity and may contribute to skin barrier functions. These findings suggest that the leaf extract may possess stronger antimicrobial and dermatological potentials. On the other hand, the pulp extract was enriched in arachidonic acid (C20:4, 4.37%), a PUFA involved in inflammatory signalling pathways and immune regulation (Calder, 2020). Although excessive arachidonic acid can drive inflammation, its balanced intake is essential for immune response and cognitive functions. Additionally, both the pulp and stem bark contained notable amounts of eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6), which are well established for cardiovascular, neuroprotective, and anti-inflammatory benefits (Swanson et al., 2012).

Gas Chromatography–Flame Ionisation Detection (GC-FID) is a highly sensitive and reproducible technique for analysing volatile and semi-volatile organic compounds (Adahchour et al., 2006). It separates complex mixtures and detects them through ionisation in a hydrogen-air flame, producing quantitative data proportional to carbon content (Snow & Slack, 2002). In drug discovery, GC-FID is

vital for identifying phytochemicals, assessing purity, and quantifying bioactive compounds such as phenolics and terpenes (Gullberg et al., 2004). Its reliability makes it valuable for standardising herbal formulations and ensuring consistency in preclinical and clinical research, thereby linking natural product chemistry with modern drug development (Gullberg et al., 2004). The GC-FID analysis of the pulp, leaf, and stembark extracts revealed distinct phytochemical distributions, with notable differences in both the qualitative and quantitative profiles of phenolic and aromatic compounds. These variations are consistent with previous findings that plant tissues often accumulate different classes of secondary metabolites depending on their physiological roles and exposure to environmental stressors (Dai & Mumper, 2010).

The pulp extract was dominated by compounds such as Hydroxyquinol (53.51 ppm), Pyrogallol (49.22 ppm), Phloroglucinol (66.20 ppm), and p-Cresol (71.95 ppm), suggesting a high abundance of polyphenolic and phenolic acid derivatives. These compounds are well known for their potent antioxidant and cytoprotective activities (Kakkar et al., 2014; Saha et al., 2011). In contrast, the leaf extract contained α -Naphthol (10.01 ppm), 3,5-Hydroxy toluene (7.61 ppm), and Picric acid (4.96 ppm) as major constituents, but at comparatively lower concentrations. This lower quantitative yield of phenolics aligns with reports that leaves, while rich in flavonoids, often show more structural diversity rather than concentration dominance (Middleton et al., 2000).

The stembark extract presented an intermediate profile, with significant levels of Quinol (29.86 ppm), Phloroglucinol (12.96 ppm), and Resorcinol (8.86 ppm). Bark tissues are typically enriched with tannins and phenolic derivatives, serving as defence compounds against microbial invasion and herbivory (Baur & Sinclair, 2006). The dominance of Quinol and Phloroglucinol in the stembark extract supports this ecological function, given their known antimicrobial and antioxidant roles (Chung et al., 2001; Lee et al., 2015).

The pulp extract exhibited the highest quantitative abundance of bioactive phenolics, particularly polyhydroxylated aromatics like Phloroglucinol and p-Cresol, which may account for stronger antioxidant and antibacterial potentials. The leaf extract, although quantitatively lower, contained unique compounds such as Xanthone and Resveratrol, associated with anticancer and cardioprotective properties (Negi et al., 2004; Baur & Sinclair, 2006). Meanwhile, the stem bark extract showed a balanced distribution of antiseptic and antimicrobial compounds, particularly Resorcinol and Quinol, aligning with its traditional use in treating infections. These results underscore that different plant parts serve complementary pharmacological roles: the pulp as a reservoir of bulk antioxidants, the leaf as a source of structurally diverse and therapeutic flavonoids, and the stem bark as a protective antimicrobial storehouse. Such partitioning of metabolites has been observed across medicinal plants, reflecting both metabolic specialisation and ecological adaptation (Zhou et al., 2016).

The proximate analysis revealed significant differences in the nutrient composition of the stem bark, leaf, and pulp extracts, highlighting their varying nutritional and physiological potentials. The moisture content was highest in the pulp (26.77%), moderate in the leaf (12.53%), and lowest in the stem bark (7.78%). High moisture levels in pulp are consistent with fruit-based tissues, which often serve as water reservoirs; however, excessive moisture may reduce shelf-life due to increased susceptibility to microbial spoilage (Ihekoronye & Ngoddy, 1985). The relatively low moisture in the stem bark suggests better storage stability. Ash content, which represents total mineral composition, was highest in the stem bark (10.47%) and leaf (10.09%), but markedly lower in the pulp (3.01%). This suggests that bark and leaves are richer sources of essential minerals such as calcium, potassium, and magnesium, which are vital for enzymatic functions and metabolic regulation (Aberoumand, 2011).

Fat and oil content was highest in the pulp (13.44%), followed by the leaf (10.42%), and lowest in the bark (4.42%). Dietary fats are important for energy storage, absorption of fat-soluble vitamins, and structural cell membrane functions (FAO/WHO, 2010). The pulp's higher oil content suggests its potential as an energy-rich food source. Fibre content was highest in the leaf (8.99%), moderate in the bark (5.24%), and lowest in the pulp (1.65%). Dietary fibre is essential for gastrointestinal health, aiding digestion, lowering cholesterol, and regulating blood sugar levels (Anderson et al., 2009). The high fibre content in leaves implies potential benefits in preventing constipation and managing metabolic disorders.

Protein content was highest in the pulp (13.30%), followed by the bark (10.02%), and lowest in the leaf (8.84%). Proteins are essential for tissue repair, enzyme synthesis, and immune function (FAO, 2011). Thus, the pulp presents higher nutritive potential in terms of protein availability. Carbohydrate content was predominant in the stem bark (62.07%), followed by the leaf (49.13%) and pulp (41.87%). Carbohydrates serve as the primary energy source, especially for brain and muscular activity (Slavin, 2013). The bark's high carbohydrate concentration suggests its role as an energy reservoir, although its bioavailability may differ due to tannins and lignins often found in bark tissues.

The vitamin composition (Table 4.7.1) varied significantly among the stem bark, leaves, and pulp extracts, reflecting their distinct nutritional and therapeutic potentials. Vitamin A content was highest in the stem bark (1.89 mg/100 g) and leaves (1.95 mg/100 g) compared to the pulp (0.55 mg/100 g). This suggests that the bark and leaves could provide better support for vision, immune function, and epithelial integrity, consistent with the role of vitamin A as a regulator of cell growth and immunity (Wiseman et al., 2017). The B-vitamins (B1, B2, B3, and B6) were present in moderate amounts across all parts, with pulp generally containing lower values except for vitamin B6, which was highest in pulp (2.43 mg/100 g). These vitamins are crucial for energy metabolism, neurotransmitter synthesis,

and redox balance (Kennedy, 2016; Kennedy, 2019). Interestingly, vitamin B12 was absent in the stem bark and leaves but recorded a significant level in pulp (4.18 mg/100 g). Since vitamin B12 is rare in plant sources, this finding could indicate microbial symbiosis or environmental enrichment, a feature recently reported in some tropical fruits (Watanabe & Bito, 2018).

Vitamin C content was highest in the pulp (6.01 mg/100 g), followed by leaves (4.56 mg/100 g) and stem bark (4.39 mg/100 g). This aligns with the common role of fruits and pulps as ascorbic acid reservoirs. Vitamin C is an essential antioxidant that enhances immune defence and collagen synthesis while reducing oxidative stress (Carr & Maggini, 2017; Mousavi et al., 2021). Vitamin D was more abundant in the leaves (9.33 mg/100 g), followed by stem bark (6.36 mg/100 g), with pulp showing the least value (0.33 mg/100 g). This is significant, as vitamin D deficiency is a global health concern linked to bone disorders, immune dysregulation, and chronic diseases (Bouillon et al., 2019; Pilz et al., 2022). The higher leaf content may be due to greater sunlight exposure, which enhances vitamin D biosynthesis.

The most striking result was the extremely high vitamin E content in stem bark (97.91 mg/100 g) and leaves (97.03 mg/100 g), compared to pulp (1.43 mg/100 g). Vitamin E is a lipid-soluble antioxidant that protects membranes from oxidative damage and has anti-inflammatory roles (Shahidi & Ambigaipalan, 2018). Recent evidence supports its role in cardiovascular protection and neuroprotection, making the bark and leaves potential sources of nutraceuticals (Maggini et al., 2018; Galli et al., 2023).

The mineral analysis (Table 4.8.1) revealed distinct differences among the stem bark, leaf, and pulp extracts, reflecting their varying nutritional and toxicological significance. Essential macronutrients such as magnesium, calcium, sodium, and potassium were detected across samples, though at different concentrations. The leaf extract was particularly rich in magnesium (9.48 ppm) and calcium (9.32

ppm), both of which are vital for enzymatic activities, neuromuscular function, and bone health (Ciosek et al., 2023). Potassium levels were also higher in the leaf (9.04 ppm), supporting its role in regulating osmotic balance, nerve transmission, and blood pressure control (Vaz-Tostes et al., 2022). Micronutrients such as zinc, iron, and selenium were also evident. Zinc was highest in the stem bark (0.96 ppm), confirming its importance in immune defence, protein metabolism, and wound healing (Skrajnowska & Bobrowska-Korczak, 2019). Iron was also abundant in the stem bark (0.46 ppm), consistent with its role in haemoglobin synthesis and oxygen transport (Abbaspour et al., 2019). Selenium, known for its antioxidant function and protection against oxidative stress, was most concentrated in the stem bark (0.72 ppm), followed by the leaf (0.61 ppm), indicating a possible protective benefit (Alkhedaide et al., 2021).

On the other hand, potentially toxic heavy metals such as lead, cadmium, mercury, and arsenic were detected in varying amounts. Lead and cadmium were present in the stem bark (0.18 and 0.19 ppm, respectively) and leaf (0.19 and 0.10 ppm, respectively), exceeding recommended safe limits for prolonged consumption and raising safety concerns (Jaishankar et al., 2014). Mercury was highest in the pulp (0.03 ppm), while arsenic was relatively low across samples. The presence of these toxic elements underscores the importance of safety evaluation and dose regulation when considering these plant parts for medicinal or nutritional applications. Trace elements such as cobalt, copper, and molybdenum also varied. The pulp showed high copper content (0.37 ppm), which is essential for enzyme activity and haemoglobin synthesis but could be toxic at elevated levels (Gaetke & Chow, 2003). Molybdenum was highest in the pulp (0.28 ppm), playing roles in nitrogen metabolism and detoxification pathways (Novotny et al., 2019). Interestingly, nickel was present in the stem bark (1.02 ppm) and pulp (0.012 ppm) but absent in the leaf, highlighting differences in mineral accumulation and possible selective uptake by plant tissues.

Antioxidants are molecules that protect biological systems by neutralising reactive oxygen and nitrogen species, thereby preventing oxidative damage to lipids, proteins, and DNA (Pisoschi & Pop, 2015). They play a critical role in maintaining redox balance, modulating inflammation, and reducing the risk of chronic diseases such as cancer, cardiovascular disorders, and diabetes (Liguori et al., 2018). Both endogenous enzymes and exogenous dietary compounds, particularly polyphenols, vitamins, and carotenoids, contribute to antioxidant defence. Plant-derived antioxidants are especially important due to their abundance and diverse bioactivities, making them key candidates in nutraceuticals and drug development (Alam et al., 2022). Nitric oxide (NO) scavenging capacity is a key antioxidant parameter because NO is a free radical that, while essential for vascular relaxation and neurotransmission, contributes to oxidative and nitrosative stress when produced in excess (Forstermann & Sessa, 2012; Marín et al., 2023). Overproduction of NO can lead to the formation of reactive nitrogen species such as peroxynitrite, which is implicated in inflammation, cardiovascular dysfunction, and tissue injury (Pisoschi & Pop, 2015; Radi, 2018). Thus, plant-derived antioxidants capable of scavenging NO are of great importance in mitigating oxidative damage and maintaining redox balance.

In this study, the pulp extract consistently demonstrated the strongest NO-scavenging activity, with the lowest residual NO concentrations across the tested doses (0.897–2.540 µg/ml). This suggests that the pulp contains a higher concentration of bioactive compounds, likely phenolics or flavonoids, with strong NO-reactive capacity (Gutiérrez-Grijalva et al., 2019; Alam et al., 2022). The stembark extract exhibited intermediate activity (1.835–2.752 µg/ml), while the leaf extract showed the weakest scavenging, reflected by the highest residual NO values (2.289–2.904 µg/ml). The observed hierarchy (Pulp > Stembark > Leaf) is consistent with previous findings that differences in phytochemical

distribution among plant parts strongly influence their antioxidant potential (Chaves et al., 2020; Tan et al., 2023).

Interestingly, a slight upward trend in residual NO values was observed as extract concentration increased, particularly in the leaf extract. This may be attributed to concentration-dependent shifts in antioxidant behaviour, where some polyphenols exert pro-oxidant effects at higher levels due to redox cycling or metal interactions (Azman et al., 2019; Gülçin, 2020). Such dual antioxidant/pro-oxidant actions are increasingly recognised as a common phenomenon in plant-derived antioxidants. The reference antioxidant, gallic acid, served as a positive control and is well known for its potent NO scavenging, anti-inflammatory, and cardiovascular protective effects (Kaur et al., 2023). Its inclusion validates the reliability of the assay and situates the extracts' activities in the broader context of polyphenolic antioxidants.

Superoxide anion ($O_2^{\bullet-}$) is a reactive oxygen species (ROS) generated during normal cellular metabolism, but its excessive accumulation leads to oxidative stress, lipid peroxidation, DNA damage, and protein oxidation (Liguori et al., 2018). Superoxide scavenging assays are therefore critical in evaluating the ability of plant extracts to neutralise this free radical, thereby reflecting their potential in preventing oxidative stress-related diseases such as cancer, diabetes, cardiovascular disorders, and neurodegeneration (Pisoschi & Pop, 2015; Chikara et al., 2018). Natural antioxidants, especially from medicinal plants, are of great pharmacological interest as safer alternatives to synthetic antioxidants in drug discovery and nutraceutical development.

At lower concentrations (10–20 mg/ml), the leaf extract demonstrated strong superoxide scavenging (65.06% at 20 mg/ml), outperforming both pulp and stem bark. This is consistent with its higher vitamin C and mineral content (magnesium, potassium, and calcium), which are essential cofactors in antioxidant defence (Sivanesan et al., 2023). The pulp extract, while weaker at 20 mg/ml, surpassed

all extracts at higher concentrations, peaking at 69.45% at 80 mg/ml, suggesting dose-dependent scavenging activity, likely linked to its elevated molybdenum and copper content, which support redox enzyme systems. The stem bark extract showed moderate activity across concentrations (54.95–67.25%), aligning with its strong vitamin E profile, a lipid-soluble antioxidant known for quenching radical chain reactions in membranes (Alam et al., 2022).

When compared to gallic acid, the extracts showed lower activity, as expected, since gallic acid is a pure phenolic antioxidant. However, the progressive increase in scavenging efficiency with higher extract concentrations suggests the presence of bioactive compounds working synergistically with vitamins and minerals to combat oxidative stress. However, the ability of the extracts to scavenge superoxide radicals highlights their therapeutic potential as natural antioxidants, especially in conditions involving oxidative stress and inflammation. The complementary roles of vitamins, minerals, and phytochemicals (previously confirmed by GC-FID and proximate analyses) indicate that these extracts can serve as effective sources of functional compounds for drug discovery and nutraceutical applications (Sies & Jones, 2020).

The hydroxyl radical ($\bullet\text{OH}$) is one of the most reactive oxygen species, capable of damaging biomolecules such as DNA, proteins, and lipids through oxidation, thereby contributing to mutagenesis, aging, and various chronic diseases (Halliwell & Gutteridge, 2015; Pizzino et al., 2017). Because of its high reactivity and short half-life, hydroxyl radical scavenging assays are a vital measure of antioxidant capacity in plant extracts. In this study, the leaf and stem bark extracts exhibited the highest hydroxyl radical scavenging activities, with maximum values of 73.034% and 73.221% at 80 mg/ml, respectively. These results were closely comparable to gallic acid, the standard phenolic antioxidant, which recorded 63.051% activity at a much lower concentration (0.005 mg/ml).

The pulp extract showed relatively lower scavenging activity (54.494–68.727%), though still significant.

The higher activity in leaf and stem bark extracts can be linked to their abundant phenolic and flavonoid constituents identified through GC-MS, as well as their richness in vitamins C and E, selenium, and zinc, which play synergistic roles in ROS neutralisation (Chikara et al., 2018; Alam et al., 2022). The pulp extract, though less potent in hydroxyl radical scavenging, demonstrated strong nitric oxide scavenging in earlier assays, suggesting a distinct but complementary antioxidant profile. The overall dose-dependent increase in hydroxyl radical scavenging suggests that the antioxidant compounds act more effectively at higher concentrations. This reinforces the extracts' potential to provide protection against oxidative DNA damage and associated pathologies such as cancer, neurodegenerative disorders, and cardiovascular diseases (Liguori et al., 2018; Sies & Jones, 2020).

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is one of the most widely used methods for evaluating antioxidant potential in plant extracts. DPPH is a stable free radical that exhibits a deep violet color, which becomes pale yellow upon reduction by antioxidants donating hydrogen or electrons (Brand-Williams et al., 1995). This makes it a simple, rapid, and reliable method for assessing free radical scavenging activity and the overall antioxidant strength of bioactive compounds (Shahidi & Ambigaipalan, 2015).

In the present study, the leaf extract demonstrated the highest DPPH radical scavenging activity across all concentrations (93.10–94.83%), nearly comparable to the synthetic antioxidant standard, BHT (98.501%). The stem bark extract showed moderate scavenging capacity (81.46–85.78%), while the pulp extract exhibited the lowest activity (75.96–80.40%). The remarkable performance of the leaf extract suggests a higher abundance of antioxidant phytochemicals such as flavonoids, tannins, and phenolic acids, as confirmed in previous phytochemical screenings. The close alignment of the leaf

extract's activity with BHT highlights its strong potential as a natural alternative to synthetic antioxidants, which are increasingly criticised for their toxicity and long-term health risks (Maqsood et al., 2014). Furthermore, the consistency of scavenging across concentrations suggests saturation at higher doses, reflecting a high efficiency of active compounds in neutralising free radicals.

These findings correlate well with the studies of other antioxidant assays (NO, superoxide, and hydroxyl scavenging), where the leaf and stem bark consistently outperformed the pulp extract. The synergy of polyphenolic compounds and vitamins likely underpins this strong radical neutralisation, making these extracts promising candidates for therapeutic and nutraceutical applications in oxidative stress-related diseases (Liguori et al., 2018; Alam et al., 2022).

Hydrogen peroxide (H_2O_2) is a reactive oxygen species (ROS) generated during normal cellular metabolism, but excessive accumulation can cause oxidative stress, leading to damage of DNA, proteins, and lipids (Sies & Jones, 2020). Although H_2O_2 itself is a weak oxidant, it can diffuse across cell membranes and, in the presence of transition metals like Fe^{2+} , generate highly reactive hydroxyl radicals via the Fenton reaction (Valko et al., 2007). Therefore, the ability of plant extracts to scavenge H_2O_2 is an important indicator of their potential to prevent oxidative damage and related chronic diseases such as cancer, diabetes, and neurodegeneration (Liguori et al., 2018).

In this study, all extracts exhibited a concentration-dependent scavenging effect against H_2O_2 . The leaf extract showed the highest scavenging activity (63.002–76.533%), outperforming the pulp (57.717–69.345%) and stem bark (55.603–65.328%). Interestingly, at higher concentrations (50–100 mg/ml), the leaf extract exhibited scavenging activity almost comparable to the reference standard, gallic acid (73.573% at 50 mg/ml). This highlights the high antioxidant potential of the leaf extract, likely due to its richness in flavonoids and phenolic acids, which are known for donating electrons to neutralise ROS (Shahidi & Ambigaipalan, 2015).

The pulp and stem bark extracts demonstrated moderate scavenging capacities, but their lower performance compared to the leaf suggests a lower concentration of active polyphenolic constituents. The relatively high effectiveness of the leaf extract in scavenging H_2O_2 aligns with previous results from DPPH, hydroxyl, and superoxide assays, consolidating the evidence that the leaf portion contains the highest concentration of bioactive antioxidant molecules. These findings further strengthen the therapeutic potential of *Artocarpus heterophyllus* extracts as natural antioxidants, supporting their possible applications in nutraceuticals and functional foods aimed at mitigating oxidative stress-related disorders (Alam et al., 2022).

The Ferric Reducing Antioxidant Power (FRAP) assay is a widely used method for evaluating the antioxidant potential of plant extracts and bioactive compounds. It measures the ability of antioxidants to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), resulting in the formation of a blue-colored ferrous–tripyridyltriazine complex, which can be quantified spectrophotometrically (Benzie & Strain, 1996). Unlike radical scavenging assays such as DPPH or hydroxyl scavenging, the FRAP assay specifically evaluates the reducing power of compounds, which reflects their electron-donating capacity—an essential mechanism of antioxidant defence in biological systems (Prior et al., 2005). In this study, all extracts demonstrated a dose-dependent increase in ferric reducing power. The stem bark extract showed the highest activity (75.882–86.530%), consistently outperforming both leaf (73.406–80.501%) and pulp (56.380–71.170%) extracts. At higher concentrations (40–80 mg/ml), the stem bark approached the reducing power of the standard antioxidant gallic acid (85.465–94.329%), highlighting its strong potential as a natural source of reducing agents.

The leaf extract also exhibited substantial reducing activity, particularly at 40–80 mg/ml (80.141–80.501%), although slightly lower than the stem bark. By contrast, the pulp extract recorded the weakest activity, suggesting a relatively lower concentration of reductive phytochemicals such as

flavonoids, phenolics, and tannins, which are key contributors to electron-donating capacity (Shahidi & Ambigaipalan, 2015). The higher FRAP values in stem bark and leaf extracts complement the trends observed in other antioxidant assays such as DPPH, hydroxyl, and hydrogen peroxide scavenging, indicating that these plant parts are rich in compounds capable of acting both as radical scavengers and as reducing agents. This dual functionality enhances their protective role against oxidative stress–induced cellular damage, which is implicated in chronic diseases including diabetes, cardiovascular disorders, and neurodegenerative conditions (Liguori et al., 2018; Alam et al., 2022).

The ABTS radical cation scavenging assay is one of the most reliable and widely applied methods for assessing the antioxidant potential of natural products. It measures the ability of antioxidants to quench the blue-green ABTS•⁺ radical cation by electron donation or hydrogen atom transfer, resulting in a decolorization proportional to the antioxidant concentration (Re et al., 1999). Compared to other assays such as DPPH, ABTS is advantageous because it can measure both hydrophilic and lipophilic antioxidant activities, providing a broader evaluation of antioxidant potential (Arnao, 2000). This makes the ABTS assay a valuable tool in assessing the radical quenching efficiency of plant extracts and bioactive compounds. In the present study, all extracts demonstrated dose-dependent ABTS radical scavenging activity, although with varying intensities. The leaf extract showed the highest activity (67.019–71.247%), followed by the stem bark (55.180–59.408%) and pulp (52.854–60.042%). In comparison, gallic acid, the standard reference antioxidant, exhibited superior scavenging activity (74.207–82.030%) across all tested concentrations.

The increasing activity with concentration suggests that the extracts contain significant levels of phenolic compounds, flavonoids, and other phytochemicals known for their ability to neutralise free radicals through electron-donating mechanisms (Shahidi & Ambigaipalan, 2015). The leaf extract's higher scavenging ability may be attributed to its richer phytoconstituent profile, which aligns with

previous findings where leaf fractions demonstrated stronger antioxidant activities in both DPPH and FRAP assays. Conversely, the pulp extract consistently showed the lowest scavenging efficiency, indicating comparatively weaker radical-quenching compounds. When compared with results from other antioxidant assays, the ABTS trend aligns with the DPPH and FRAP outcomes, where the leaf and stem bark extracts exhibited greater activity than the pulp. This consistency across multiple assays underscores the strong antioxidant potential of leaf and stem bark, suggesting they are the richest in compounds capable of neutralising reactive species. Such activity is of physiological importance because ABTS scavenging reflects the extracts' potential to counteract free radicals implicated in oxidative stress, inflammation, and the onset of chronic diseases such as cardiovascular disorders, neurodegenerative diseases, and diabetes (Liguori et al., 2018; Alam et al., 2022).

The present study successfully established a testosterone propionate-induced model of benign prostatic hyperplasia (BPH) in adult male albino rats, as confirmed by the biochemical, macroscopic, and histological assessments.

Subcutaneous administration of testosterone resulted in a statistically significant elevation of serum prostate-specific antigen (PSA) levels in the untreated control group when compared to the normal control. This observation is consistent with the findings of Li, Wang, Guo, & Zhang, (2018), who reported that the rat prostate exhibits a pronounced response to testosterone-induced benign prostatic hyperplasia (BPH). The increase in serum PSA levels observed in this study serves as a reliable biomarker indicating the successful induction of BPH. PSA is secreted by the epithelial cells of the prostate gland, and its abnormal elevation in serum is widely recognized as a clinical indicator of prostatic dysfunction (Velonas, Woo, dos Remedios, & Assinder, 2013). Therefore, the testosterone-mediated rise in PSA levels provides strong evidence of prostatic alteration and supports the validity of the experimental model employed.

The morphological differences observed between control and treated groups provide compelling evidence of androgen-driven prostatic enlargement, aligning with previous literature on experimental BPH models. Prostates excised from testosterone-treated groups (B and C) exhibited visible hypertrophy, increased vascularity, and darker coloration compared to the control group (A), which retained normal architecture. These gross anatomical changes are consistent with the findings of Suvarna , Obisike, & Eze. (2019), who reported similar macroscopic features following TP administration in rats.

Previous studies have shown that TP-induced BPH is characterized by epithelial hyperplasia, stromal thickening, and reduced glandular lumen space (Lee et al., 2011). These microscopic features are driven by the conversion of testosterone to dihydrotestosterone (DHT), which binds to androgen receptors in the prostate and stimulates cellular proliferation. The observed anatomical changes in group B is therefore consistent with the pathophysiological response to exogenous androgen stimulation.

The successful induction of BPH in this study set a robust foundation for evaluating the therapeutic efficacy of jackfruit tree extracts. Given the observed and documented antioxidant, anti-inflammatory, and phytochemical properties of *A. heterophyllus* stem bark, leaves and pulp ethanolic extract and their potential to attenuate androgen-induced prostatic hyperplasia .

The in vivo antioxidant profile provides crucial insights into the protective effects of plant extracts against oxidative stress by measuring key biomarkers of oxidative damage and enzymatic/non-enzymatic defence systems.

Malondialdehyde (MDA) is a product of lipid peroxidation and a major marker of oxidative stress. Elevated levels reflect increased reactive oxygen species (ROS)-induced membrane damage (Del Rio

et al., 2020). The negative control showed significantly higher MDA (3.16 ± 0.20), indicating oxidative stress, whereas treatments, especially stem (500 mg: 1.98 ± 0.02) and pulp (500 mg: 1.97 ± 0.02), reduced MDA close to normal control, suggesting strong lipid peroxidation inhibition. Reduced glutathione (GSH) is a pivotal intracellular antioxidant that detoxifies ROS directly or via glutathione peroxidase (Vučković et al., 2022). The negative control showed the lowest GSH (7.96 ± 0.17), reflecting oxidative depletion. Extracts, particularly stem (500 mg: 12.16 ± 0.23) and combination (500 mg: 11.29 ± 1.31), restored GSH to levels comparable with the control (12.29 ± 0.36), indicating enhanced cellular redox defence.

Superoxide dismutase (SOD) is the first-line enzymatic antioxidant that catalyses the dismutation of superoxide radicals into hydrogen peroxide (Zhou et al., 2023). Negative control animals exhibited reduced SOD (19.84 ± 1.09), while treatments, especially combination (500 mg: 33.22 ± 1.87) and control (34.88 ± 0.24), showed significant recovery. This suggests synergistic phytoconstituents in the extracts effectively upregulated antioxidant enzymes. Catalase (CAT) complements SOD by decomposing hydrogen peroxide into water and oxygen, preventing hydroxyl radical formation (Akinmoladun et al., 2020). The negative control exhibited the lowest CAT (2.04 ± 0.11), while extracts such as pulp (500 mg: 3.10 ± 0.06), stem (500 mg: 3.05 ± 0.09), and combination (250–500 mg: 3.16 – 3.21) enhanced CAT activity, approaching or surpassing the control (3.34 ± 0.15).

Overall, the stem extract at 500 mg showed the most consistent effect across all parameters, significantly reducing lipid peroxidation while enhancing both enzymatic (SOD, CAT) and non-enzymatic (GSH) antioxidant systems. The combination extract also demonstrated strong synergistic efficacy, particularly in SOD and CAT recovery. The pulp extract, though less potent in GSH elevation, effectively reduced MDA and boosted CAT, suggesting selective antioxidant mechanisms. These findings corroborate earlier *in vitro* antioxidant assays (DPPH, FRAP, ABTS), where the leaf

and stem extracts consistently displayed superior free radical scavenging compared to the pulp. In vivo, however, the stem and combination extracts outperformed, suggesting possible differences in bioavailability, metabolism, and synergistic interactions in biological systems. Importantly, restoration of antioxidant enzyme activities and reduction of oxidative stress markers highlight the therapeutic potential of these extracts in mitigating oxidative damage linked to chronic diseases (Chedea et al., 2022; Muriithi et al., 2023).

The antioxidant activity observed in the leaf, pulp, and stem bark extracts complements their nutritional and phytochemical composition. The leaf extract consistently showed higher nitric oxide (NO) scavenging activity, particularly at 20–80 mg/ml, aligning with its elevated vitamin C, vitamin D, and mineral concentrations such as magnesium, calcium, and potassium, all known to support redox balance and enzymatic antioxidant functions (Sivanesan et al., 2023; Liguori et al., 2018). The pulp extract, although lower in most vitamins, exhibited moderate antioxidant activity, likely influenced by its high vitamin B12 and molybdenum content, which are cofactors in redox reactions. Stem bark extract demonstrated intermediate activity, correlating with its high vitamin E levels, a potent lipid-soluble antioxidant (Alam et al., 2022).

The results indicate that antioxidant potential is not solely dependent on a single nutrient but on a synergistic interplay between vitamins (C and E), minerals (selenium, zinc, and manganese), and bioactive phytochemicals detected in the GC-FID and proximate analyses. This suggests that the extracts could play complementary roles in mitigating oxidative stress through both enzymatic and non-enzymatic pathways, reinforcing their value in nutraceutical and drug discovery applications (Pisoschi & Pop, 2015; Chikara et al., 2018).

Prostate-specific antigen (PSA), testosterone, tumour necrosis factor-alpha (TNF- α), and interleukins are critical biomarkers for assessing prostate health, inflammation, and cancer progression. Elevated

PSA levels are strongly associated with prostate enlargement and malignancy risk (Loeb et al., 2021). Similarly, dysregulated testosterone levels influence prostate cell proliferation, while increased TNF- α and interleukins reflect chronic inflammation, which drives carcinogenesis (Sfanos & De Marzo, 2022). In this study, the negative control group showed significantly higher testosterone (3.44 ng/mL), PSA (3.10 ng/mL), TNF- α (25.22 pg/mL), and interleukin (85.30 pg/mL) compared to the control, confirming induced prostate dysfunction. Finasteride (5 mg), the standard drug, significantly reduced PSA and inflammatory markers, aligning with its role in suppressing androgen-driven prostate growth (Schaeffer et al., 2022).

Among the extracts, stem bark (500 mg) demonstrated the most comparable effect to finasteride, reducing testosterone (0.94 ng/mL), PSA (1.53 ng/mL), TNF- α (19.17 pg/mL), and interleukin (60.25 pg/mL). This suggests potent anti-androgenic and anti-inflammatory properties. Leaf and pulp extracts also showed dose-dependent reductions in markers, though less effective than stem bark. Interestingly, the extract combination (500 mg) produced synergistic effects, lowering PSA (1.48 ng/mL) and TNF- α (17.25 pg/mL) close to control levels, while balancing cytokine suppression. Overall, the results highlight that bioactive phytochemicals in the extracts modulate androgen signalling and inflammatory cascades, thereby improving prostate health. The stem bark and extract combination was particularly effective, suggesting possible use as complementary therapeutic agents.

Liver enzymes such as ALT (alanine aminotransferase), AST (aspartate aminotransferase), ALP (alkaline phosphatase), and LDH (lactate dehydrogenase) are critical biomarkers for assessing hepatic function, oxidative stress, and tissue damage. Elevated levels typically signal hepatocellular injury or impaired metabolism, while restoration toward normal values indicates protective or therapeutic effects (Wu et al., 2022). The negative control group exhibited significantly elevated ALT, AST, and ALP levels compared to the control, suggesting liver injury and oxidative stress likely induced by the

pathological condition. This finding is consistent with the established role of elevated transaminases as markers of hepatocyte damage (Wang et al., 2023).

Treatment with leaves (250–500 mg) moderately reduced ALT and AST compared to the negative control but remained higher than the control, suggesting partial hepatoprotection. Interestingly, leaf extract at 500 mg showed a marked increase in LDH (163.20 U/L), suggesting potential metabolic strain despite moderate enzyme regulation. The stem extracts (250–500 mg) produced the most pronounced protective effect, with ALT, AST, and ALP levels comparable to the control. Particularly, stem (500 mg) restored ALT (15.16 U/L) and AST (12.88 U/L) close to normal, demonstrating superior hepatoprotective activity. This aligns with reports that plant phenolics and flavonoids modulate transaminase activities and stabilise hepatocyte membranes (Zhang et al., 2024). The pulp extracts showed moderate enzyme regulation but were less effective than stem and leaf extracts, while finasteride produced only partial improvements, suggesting that phytochemical-rich extracts had stronger effects on hepatoprotection.

The combination extract (500 mg) performed comparably to the stem extracts, normalising ALT, AST, and ALP while keeping LDH stable. This indicates possible synergistic interactions among phytochemicals across plant parts. The results suggest that the extracts—especially stem and combination treatments—exert hepatoprotective effects by modulating liver enzyme activities, reducing oxidative stress, and stabilising cellular integrity. Such findings support the therapeutic potential of natural antioxidants in liver protection, consistent with emerging evidence on plant-derived bioactives in hepatoprotection (Li et al., 2021; Zhao et al., 2023).

Bilirubin (total and direct) is a major marker of hepatic function, as its accumulation indicates impaired bilirubin metabolism or excretion, often associated with hepatocellular damage or cholestasis (Kim et al., 2023). Serum proteins, particularly albumin and globulin, reflect both the

synthetic ability of the liver and immune status. Albumin levels indicate nutritional and hepatic synthetic capacity, while globulins reflect inflammatory and immune processes (Gao et al., 2022). Thus, assessment of bilirubin and proteins together provides a holistic understanding of liver integrity and systemic response.

The negative control group exhibited significantly elevated total (24.40 $\mu\text{mol/L}$) and direct bilirubin (17.21 $\mu\text{mol/L}$) compared to the control group, confirming hepatic dysfunction. Concurrently, total protein (5.29 g/dL), globulin (1.88 g/dL), and albumin (3.76 g/dL) were markedly reduced, suggesting impaired synthetic capacity. Treatment with extracts, finasteride, or their combinations markedly reduced bilirubin levels and improved protein synthesis. For instance, stem bark at 500 mg restored bilirubin values close to control (15.31 $\mu\text{mol/L}$ total; 12.54 $\mu\text{mol/L}$ direct), with the highest recovery in total protein (9.39 g/dL) and albumin (5.19 g/dL). Similarly, leaf and pulp extracts improved parameters in a dose-dependent manner, although pulp extracts showed comparatively lower protein recovery (6.65–7.54 g/dL).

Interestingly, the extract combination (500 mg) achieved a balance with moderately reduced bilirubin (16.50 $\mu\text{mol/L}$) and relatively restored proteins (8.66 g/dL total protein; 4.88 g/dL albumin), suggesting possible synergism. These results demonstrate that the plant extracts, especially stem bark and combined formulations, exhibit hepatoprotective activity by restoring bilirubin metabolism and enhancing protein synthesis. Elevated bilirubin and decreased proteins in untreated groups confirm liver damage, while normalisation by extracts highlights therapeutic potential, aligning with recent studies emphasising natural hepatoprotective compounds (Zhang et al., 2023; Hassan et al., 2024).

Kidney function biomarkers and electrolyte balance provide crucial insight into renal integrity and systemic homeostasis. Urea and creatinine are primary indicators of renal clearance and glomerular function, while electrolytes such as sodium, potassium, chloride, and bicarbonate regulate fluid

balance, neuromuscular function, and acid–base homeostasis (Kazancıoğlu, 2023; Chewcharat & Thongprayoon, 2020). In this study, the negative control group exhibited significantly elevated urea (30.43 mg/dL) and creatinine (7.27 mg/dL) compared to the control, suggesting renal impairment. This was also accompanied by electrolyte imbalance, with sodium (187.20 mmol/L) and chloride (81.77 mmol/L) markedly higher, and bicarbonate (14.34 mmol/L) significantly lower. These alterations are indicative of compromised kidney function and metabolic acidosis (Nagai et al., 2022). Finasteride treatment and plant extract groups generally ameliorated these abnormalities. Notably, stem extracts (500 mg) restored creatinine (5.34 mg/dL) and bicarbonate (16.86 mmol/L) close to control levels, while maintaining stable sodium and potassium concentrations. Similarly, the combination extract (500 mg) maintained bicarbonate (20.71 mmol/L) within the physiological range, suggesting possible nephroprotective effects.

On the other hand, leaf and pulp extracts showed moderate improvements, with slightly elevated urea but generally normalised electrolytes, implying dose-dependent effects. Interestingly, potassium levels remained within a narrow physiological range across groups, which is essential since both hypokalemia and hyperkalemia can be life-threatening (Khitan & Agarwal, 2021). The results indicate that the extracts, particularly the stem and combination formulations, exhibit nephroprotective properties by mitigating urea and creatinine elevation and maintaining electrolyte balance. This supports the hypothesis that phytochemicals in these plant parts contribute to renal protection, possibly via antioxidant and anti-inflammatory mechanisms (Ghosh et al., 2022).

Haematological parameters provide essential insight into immune function and systemic health. White blood cells (WBCs) are the primary immune defence cells, while lymphocytes (LYM) are critical for adaptive immunity, and monocytes (MON) play key roles in phagocytosis and inflammation regulation (Suresh et al., 2021; Kumar et al., 2020). In this study, the negative control group exhibited

elevated lymphocyte counts (54.30%) compared to the control (27.67%), suggesting immune activation, possibly due to induced stress or inflammatory responses. Monocyte levels, however, remained similar to control (7.67%), indicating selective immune modulation. Finasteride treatment and some plant extracts, particularly stem and pulp extracts at 250 mg, elevated monocyte levels (18–19%), potentially enhancing innate immune defence, while moderating lymphocyte overactivation. Conversely, higher doses of leaves and pulp (500 mg) reduced lymphocyte counts (20–22%), approaching normal control levels, which may reflect immunomodulatory effects of bioactive compounds. The combined extract exhibited balanced WBC, lymphocyte, and monocyte levels, suggesting synergistic immunoregulatory effects. Overall, the results indicate that the plant extracts can modulate both innate and adaptive immunity, supporting their potential role in improving systemic immune resilience (Moura et al., 2022).

Full blood count (FBC) is a cornerstone in evaluating the haematological status of experimental subjects. It provides crucial insights into erythropoiesis, oxygen transport capacity, immune competence, and hemostasis (Cieslak et al., 2021). Key parameters include haemoglobin (HB), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and platelets (PLT). Variations in these parameters reflect physiological adaptations or pathological perturbations resulting from dietary components, bioactive compounds, or drug administration (Gupta et al., 2022).

In this study, HB ranged from 14.10 ± 0.56 g/dL in the negative control to 18.30 ± 2.39 g/dL in the 500 mg pulp group, while RBC ranged from $11.00 \pm 2.00 \times 10^6/\mu\text{L}$ in the 500 mg leaves group to $14.80 \pm 3.20 \times 10^6/\mu\text{L}$ in the 500 mg combination extract group. These values indicate that all treatments maintained haemoglobin and RBC levels within the normal physiological range, suggesting no hematotoxic effects. Notably, stem and pulp extracts at both doses slightly elevated HB

and RBC compared to the negative control. This increase may be linked to the rich protein, iron, and micronutrient content (e.g., magnesium, zinc, and B vitamins) observed in the proximate, vitamin, and mineral analyses, which support erythropoiesis and red cell stability (Gupta et al., 2022; Cieslak et al., 2021). MCV (35.50–37.03 fL), MCH (11.30–14.80 pg), and MCHC (31.30–41.33%) values remained consistent across all treatments. These parameters reflect red blood cell size and haemoglobin content, indicating that the extracts did not induce macrocytosis, microcytosis, hypochromia, or hyperchromia. Consistent MCV, MCH, and MCHC values suggest that the bioactive compounds in leaves, stem, and pulp extracts support normal erythrocyte morphology, possibly through antioxidant-mediated protection of cell membranes from oxidative stress (Zhou et al., 2020). Platelet counts ($8806\text{--}9933 \times 10^3/\mu\text{L}$) were stable across all groups, showing that the extracts did not negatively impact thrombopoiesis or hemostatic function. Maintaining platelet levels is crucial for coagulation, especially in conditions where oxidative stress or inflammation could impair platelet production (Vera et al., 2021). The observed haematological improvements likely correlate with the antioxidant properties previously discussed (DPPH, ABTS, FRAP, superoxide scavenging), as oxidative stress can impair erythrocyte lifespan and function. Antioxidants from the extracts may protect red blood cell membranes from lipid peroxidation, reduce hemolysis, and support bone marrow erythropoiesis (Sharma et al., 2022). Additionally, micronutrients such as iron, zinc, and B-vitamins in the extracts are essential cofactors for haemoglobin synthesis and RBC maturation (Gupta et al., 2022).

The FBC outcomes complement kidney and liver function analyses, where extracts maintained normal urea, creatinine, bilirubin, and liver enzyme levels, indicating systemic safety. The haematological safety further corroborates the *in vivo* antioxidant and anti-inflammatory results, suggesting that the extracts do not induce hematopoietic toxicity while potentially enhancing red blood cell resilience and

immune function (Vera et al., 2021). Maintaining or enhancing HB, RBC, and platelet counts is critical for conditions like anaemia, chronic inflammation, or oxidative stress-related disorders. The findings suggest that these plant extracts, particularly the stem and pulp, may serve as natural hematopoietic-supportive agents, potentially improving oxygen transport, energy metabolism, and overall physiological resilience (Sharma et al., 2022).

The histopathological findings from this study provide compelling evidence of the therapeutic potential of ethanol extracts of *Artocarpus heterophyllus* (jackfruit) in ameliorating tissue damage associated with testosterone propionate (TP)-induced benign prostatic hyperplasia (BPH). The liver, kidney, and prostate—organs susceptible to oxidative stress, hormonal imbalance, and inflammatory insults—demonstrated clear patterns of damage in untreated BPH rats and marked improvements in treated groups.

The liver is particularly vulnerable to oxidative stress and mitochondrial dysfunction triggered by exogenous testosterone administration. The severe degeneration, necrosis, and central vein dilation observed in the untreated BPH group (Plate 4.4) are consistent with reports that TP induces hepatocellular injury via lipid peroxidation, mitochondrial impairment, and proinflammatory cytokine activation (Nishiyama et al., 2007). Treatment with Finasteride and especially ethanol extracts of *A. heterophyllus* (Plates 4.8–4.12) ameliorated these alterations, with the pulp and combined extracts restoring near-normal hepatocytes and portal triads. These hepatoprotective effects can be attributed to phytochemicals such as flavonoids, phenolic acids, stilbenoids, and tannins, which enhance antioxidant enzyme activity, scavenge reactive oxygen species (ROS), and stabilise hepatocyte membranes (Rahman et al., 2019; Singh et al., 2021). Similar outcomes have been reported in chemically induced liver injury, where jackfruit extracts reduced serum ALT, AST, ALP, and bilirubin while preserving lobular architecture (Nwabueze et al., 2022). The mild degeneration noted

in high-dose stem bark and leaf extracts suggests potential cytotoxicity at elevated concentrations, likely due to excess tannins or alkaloids, reinforcing the importance of dose optimisation.

The kidney histology corroborated these hepatoprotective trends. Untreated BPH rats exhibited severe tubular epithelial degeneration and necrosis (Plate 4.15), features consistent with androgen-induced nephrotoxicity (Xia et al., 2013). By contrast, treatment with stem bark and pulp extracts at both doses (Plates 4.17–4.23) preserved normal renal histoarchitecture, including intact glomeruli and tubules, demonstrating nephroprotective efficacy. This protective effect is likely mediated by the antioxidant and anti-inflammatory actions of jackfruit phytoconstituents, which upregulate catalase and glutathione peroxidase activity, reducing tissue necrosis (Islam et al., 2020). However, the leaf extract at 500 mg/kg and the high-dose combination extract (Plates 4.20 and 4.24) induced tubular necrosis, highlighting a biphasic, hormetic response (Calabrese et al., 2010). While moderate doses enhanced renal defence, excessive phytochemical concentrations disrupted redox balance, underlining the nuanced dose–response dynamics in phytotherapy.

The prostate gland provided the most direct evidence of therapeutic efficacy. The untreated BPH group (Plate 4.26) exhibited classical features of hyperplasia: epithelial hypertrophy, luminal projections, and increased secretory activity, all indicative of androgen-driven proliferation (Carson & Rittmaster, 2003). Finasteride treatment partially reversed these changes (Plate 4.27), consistent with its 5 α -reductase inhibitory effect but limited by incomplete androgen suppression. Notably, *A. heterophyllus* extracts, particularly at higher doses of stem bark, pulp, and combined fractions (Plates 4.29–4.35), markedly improved acinar and epithelial architecture, restoring near-normal histology. Phytosterols and flavonoids in jackfruit may act as natural 5 α -reductase inhibitors (Liu et al., 2015), modulating androgen receptor signalling while providing anti-inflammatory and antioxidant support

(Sharma et al., 2017). This multifaceted activity likely accounts for the normalisation of epithelial thickness and reduced secretory hyperactivity.

Conversely, incomplete resolution of hyperplasia was noted in groups treated with low-dose stem bark and pulp extracts (Plates 4.28 and 4.32) as well as high-dose leaf extract (Plate 4.31), with persistent epithelial hypertrophy and luminal projections. These outcomes suggest that therapeutic efficacy depends on phytoconstituent balance and concentration across different plant parts, with pulp and combined extracts demonstrating the most consistent protective profiles.

When histopathological findings were compared across treatment groups, clear dose- and organ-dependent responses emerged. In the kidney, Groups 1, 4, 5, 6, 8, 9, and 10 preserved normal renal structures, while Groups 2 and 7 showed severe degeneration, and Groups 3 and 11 displayed mild to moderate damage. Similarly, the liver in Groups 1, 6, 8, and 9 appeared normal, while severe degeneration was evident in Groups 2 and 7; moderate injury occurred in Groups 3, 4, 5, and 11. These structural differences correlated with biochemical markers such as ALT, AST, ALP, LDH, and bilirubin (Santos et al., 2021; Lee & Park, 2023). Prostate histology showed normal acini in Groups 1, 5, 6, 9, and 10, whereas Groups 2, 3, 4, 7, and 8 displayed epithelial thickening and projections, aligning with elevated serum testosterone, PSA, TNF, and interleukin levels (Kumar et al., 2022; Santos et al., 2021).

The histopathological outcomes support the biochemical and haematological data from this and related studies. Extract-treated groups demonstrated reductions in PSA, liver enzymes, and renal biomarkers alongside improvements in antioxidant enzyme status (Rahman et al., 2019; Singh *et al.*, 2021). Improvements in lipid profiles, such as reduced cholesterol and triglycerides, further contribute to prostate health, as dyslipidemia is linked with BPH progression (Sharma et al., 2017). The

consistency between preserved tissue morphology, normalised biochemical parameters, and modulated inflammatory markers underscores the systemic therapeutic potential of *A. heterophyllus*.

These findings suggest that ethanol extracts of *A. heterophyllus* exert hepatoprotective, nephroprotective, and anti-BPH effects through a combination of anti-androgenic, antioxidant, anti-inflammatory, and metabolic mechanisms. However, the biphasic, dose-dependent responses observed highlight the need for cautious dosing and plant-part selection to maximise therapeutic efficacy while minimising cytotoxic risks (Calabrese et al., 2010; Lee & Park, 2023; Santos et al., 2021).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The comprehensive phytochemical, nutritional, and *in vivo* evaluation of *Artocarpus heterophyllus* leaf, stem bark, and pulp extracts demonstrates that each plant part offers distinct yet complementary therapeutic and nutraceutical benefits. GC–MS, GC-FID, FTIR, and proximate analyses reveal tissue-specific distributions of bioactive compounds, including fatty acids, phenolics, flavonoids, nitrogen- and sulfur-containing compounds, and vitamins. The stem bark is particularly rich in oleic acid, α -linolenic acid, proline, and fat-soluble vitamins, supporting antioxidant, anti-inflammatory, antimicrobial, cardiovascular, and wound-healing applications. The leaf extract, enriched in threonine, isoleucine, arginine, polyunsaturated fatty acids, and essential minerals, exhibits strong

radical-scavenging, immune-modulating, and antimicrobial properties. The pulp, although less concentrated in secondary metabolites, contains polyhydroxylated phenolics, neurotransmitter precursors, and PUFAs such as DHA and EPA, highlighting neuromodulatory, cardioprotective, and nutritional potential. Together, these findings suggest a partitioning of bioactive functions: the pulp acts as a bulk antioxidant and nutrient reservoir, the leaf provides structurally diverse therapeutic metabolites, and the stem bark serves as a defensive and organ-protective phytochemical source.

In vivo assessments confirm that these extracts confer significant hepatoprotective, nephroprotective, hematopoietic, immunomodulatory, antioxidant, and prostate-supportive effects. The stem bark and combination extracts consistently normalized liver enzymes (ALT, AST, ALP, LDH), bilirubin, protein synthesis, kidney markers (urea, creatinine, electrolytes), and oxidative stress indicators (MDA, GSH, SOD, CAT). They also modulated testosterone, PSA, TNF- α , and interleukin levels, reflecting anti-androgenic and anti-inflammatory activities, while maintaining normal haematological parameters. Leaf and pulp extracts demonstrated moderate, dose-dependent benefits, highlighting tissue-specific efficacy and bioavailability. Histopathological findings corroborate these biochemical outcomes, showing preserved liver, kidney, and prostate architecture in treated groups. Overall, the protective effects of *A. heterophyllus* are underpinned by the synergistic interplay of vitamins (C, D, E), minerals (selenium, zinc, magnesium), and phytochemicals (phenolics, flavonoids), which collectively mitigate oxidative stress, inflammation, and tissue damage.

These findings underscore the importance of integrating traditional knowledge with modern scientific methods to explore the full medicinal potential of plants, and as research continues to evolve, medicinal plants remain a promising source for the discovery of new treatments for various health conditions.

5.2. Recommendations

Given the results from this study, we, therefore recommend that, Clinical trials are warranted to validate the safety, bioavailability, and efficacy of *A. heterophyllus* extracts in humans, while careful dose titration is recommended to maximize therapeutic benefits and avoid potential metabolic or immunomodulatory strain, particularly with higher doses of leaf extracts. Additionally, isolation and characterization of key phytochemicals could inform drug development and the design of functional nutraceutical.

5.3. Contribution to Knowledge

The findings of this study contribute to scientific knowledge in the following ways:

1. It provides compelling biochemical evidence for the therapeutic potential of ethanol extracts from *A. heterophyllus* (jackfruit), particularly the stem and combination formulations (ethanol extract of stem bark, leaves and fruit pulp).
2. It advances knowledge by demonstrating that different parts of *A. heterophyllus* (stem bark, pulp, and leaves) possess distinct phytochemical profiles that translate into varying biological activities. The GC–MS analysis revealed tissue-specific secondary metabolites, with the pulp showing the greatest chemical diversity and the stem bark being particularly rich in vitamin E and fatty acids, both correlating with strong antioxidant and hepatoprotective effects.
3. By integrating in vitro assays, in vivo biochemical markers, and histopathological evaluations, this work provides the first comprehensive evidence that *A. heterophyllus* extracts not only attenuate oxidative stress but also exert nephroprotective, hepatoprotective, and hematomodulatory effects. The consistency between phytochemical composition and bioactivity underscores the pharmacological potential of this plant as a natural source of therapeutic agents. Furthermore, the comparative analysis

across plant parts offers new insights into tissue-specific efficacy, suggesting that the pulp and stem bark are particularly promising candidates for drug development.

REFERENCES

- Abbaspour, N., Hurrell, R., & Kelishadi, R. (2019). Review on iron and its importance for human health. *Journal of Research in Medical Sciences*, 19(2), 164–174. <https://doi.org/10.4103/1735-1995.132472>.
- Aberoumand, A. (2011). Preliminary assessment of proximate composition of plant foods commonly used in Iran. *Nutrition & Food Science*, 41(1), 68–76. <https://doi.org/10.1108/00346651111102719>.
- Achika, J. I., Ayo, R. G., Khan, E. M., & Shehu, A. (2023). Identification of antioxidant compounds from the stem bark fraction of *Strychnos innocua* Del. *Journal of Agriculture and Food Research*, 14, 100833.
- Adahchour, M., Beens, J., Vreuls, R. J. J., & Brinkman, U. A. T. (2006). Recent developments in comprehensive two-dimensional gas chromatography (GC × GC) II. Modulation and

- detection. *TrAC — Trends in Analytical Chemistry*, 25(6), 540–553. <https://doi.org/10.1016/j.trac.2006.04.004>.
- Adewole, K. E., Salami, S. A., Akinmoladun, F. O., Olaleye, T. M., Komolafe, T. R., & Olasehinde, T. A. (2018). GC-MS analysis and pharmacological potentials of bioactive compounds identified in the methanolic leaf extract of *Senecio biafrae*. *Journal of Applied Sciences and Environmental Management*, 22(2), 245–250. <https://doi.org/10.4314/jasem.v22i2.16>.
- Adlercreutz, H. (2007). Lignans and human health. *Critical Reviews in Clinical Laboratory Sciences*, 44(5–6), 483–525. <https://doi.org/10.1080/10408360701612942>.
- Agunloye, M. O., Owu, D. U., Onaadebo, O., Ugwu, F. N., & Ogunyemi, O. M. (2025). Phytochemical characterization of ethanolic and ethyl acetate extracts of avocado *Persea americana* leaves by FT-IR and GC-MS reveals potential bioactive compounds. *Scientific Reports*, 15(1), 27035. <https://doi.org/10.1038/s41598-025-12150-z>.
- Ajayi, O. A. (2021). Phytochemical and GC-MS analysis of bioactive components in ethanolic extract of *Rauvolfia vomitoria* leaves. *Journal of Chemical Society of Nigeria*, 46(4).
- Ajayi, O. S., & Akinsanya, M. A. (2023). Phytochemical analysis and bioactive potential of fatty acids isolated from medicinal plants. *Journal of Medicinal Plant Research*, 17(4), 112–121. <https://doi.org/10.5897/JMPR2023.7211>.
- Akamphon, S., Akamphon, K. (2014). Cost and benefit tradeoffs in using a shade tree for residential building energy saving. *Environment Asia*, 7 (1): 19-24. <http://dx.doi.org/10.14456/ea.2014.4>
- Akinmoladun, F. O., Komolafe, T. R., Farombi, O. E., & Oyedapo, O. O. (2020). Phytochemical and pharmacological activities of secondary metabolites from plants: Implications for drug discovery. *Pharmacognosy Reviews*, 14(28), 33–46. https://doi.org/10.4103/phrev.phrev_32_20.
- Akinmoladun, F. O., Komolafe, T. R., Olaleye, T. M., Farombi, E. O., & Oyedapo, O. O. (2020). Natural products as modulators of endogenous antioxidant defense in oxidative stress and diseases. *Archives of Physiology and Biochemistry*, 126(3), 192–202. <https://doi.org/10.1080/13813455.2018.1540985>.
- Alabi, O. A., Awosode, O. D., & Olayemi, F. O. (2018). Oleic acid and related fatty acids: Pharmacological activities and therapeutic implications. *African Journal of Biochemistry Research*, 12(5), 58–66. <https://doi.org/10.5897/AJBR2018.9674>.
- Alam, M. N., Almoyad, M., & Huq, F. (2022). Polyphenols in the prevention and treatment of vascular and cardiovascular disease: A review. *Molecules*, 27(18), 5991. <https://doi.org/10.3390/molecules27185991>.
- Alamoudi, A. J., Alessi, S. A., Rizg, W. Y., Jali, A. M., Safhi, A. Y., Sabei, F. Y., Alshehri, S., Hosny, K. M., & Abdel-Naim, A. B. (2022). Cordycepin Attenuates Testosterone-Induced Benign

- Prostatic Hyperplasia in Rats via Modulation of AMPK and AKT Activation. *Pharmaceutics*, 14(8), 1652. <https://doi.org/10.3390/pharmaceutics14081652>
- Albratty, M., Alhazmi, H. A., Meraya, A. M., Najmi, A., Alam, M. S., Rehman, Z., & Moni, S. S. (2021). Spectral analysis and antibacterial activity of the bioactive principles of *Sargassum tenerrimum* J. Agardh collected from the Red Sea, Jazan, Kingdom of Saudi Arabia. *Brazilian Journal of Biology*, 83. <https://doi.org/10.1590/1519-6984.249536>.
- Ali, S. I., Gopalakrishnan, B., & Venkatesalu, V. (2014). Pharmacological activities of picric acid. *Journal of Applied Pharmaceutical Science*, 4(7), 92–96. <https://doi.org/10.7324/JAPS.2014.40717>.
- Alkhedaide, A., Alshehri, Z. S., Sabry, A., Abdel-Ghaffar, T., Soliman, M. M., & Shehata, S. M. (2021). Protective role of selenium against oxidative stress and inflammation. *Biological Trace Element Research*, 199(11), 4115–4125. <https://doi.org/10.1007/s12011-020-02494-7>.
- Al-Salman, H. N. K. (2019). Antimicrobial activity of the compound 2-Piperidinone, N-[4-Bromo-n-butyl]-extracted from pomegranate peels. *Asian Journal of Pharmaceutics (AJP)*, 13(01). <https://doi.org/10.22377/ajp.v13i01.3008>
- American Academy of Family Physicians. (2023). *Benign prostatic hyperplasia: Rapid evidence review*. <https://www.aafp.org/pubs/afp/issues/2023/0600/benign-prostatic-hyperplasia.pdf>
- Amin, M.B., Tickoo, S.K. (2022) Diagnostic Pathology: Genitourinary E-Book. Elsevier Health Sciences.
- Anderson, J. W., Baird, P., Davis, R. H., Ferreri, S., Knudtson, M., Koraym, A., Waters, V., & Williams, C. L. (2009). Health benefits of dietary fiber. *Nutrition Reviews*, 67(4), 188–205. <https://doi.org/10.1111/j.1753-4887.2009.00189.x>.
- AOAC. (1995). Official methods of analysis 16th Ed. Association of official analytical chemists. Washington DC, USA.
- Aparna, V., Dileep, K. V., Mandal, P. K., Karthe, P., Sadasivan, C., & Haridas, M. (2012). Anti-inflammatory property of n-hexadecanoic acid: Structural evidence and kinetic assessment. *Chemical Biology & Drug Design*, 80(3), 434–439. <https://doi.org/10.1111/j.1747-0285.2012.01418.x>.
- Araújo, N. G., & Lima, L. R. P. (2010). Utilization of *Artocarpus heterophyllus* in the treatment of calcium oxalate stones. *Infarma*, 22(11-12), 3-7.
- Arnao, M. B. (2000). Some methodological problems in the determination of antioxidant activity using chromogen radicals: A practical case. *TrAC Trends in Analytical Chemistry*, 19(10), 617–628. [https://doi.org/10.1016/S0165-9936\(00\)00068-2](https://doi.org/10.1016/S0165-9936(00)00068-2)

- Asghari, G., Jalali, M., & Sadoughi, E. (2012). Antimicrobial activity and chemical composition of essential oil from the seeds of *Artemisia aucheri* Boiss. *Jundishapur Journal of Natural Pharmaceutical Products*, 7(1), 11–15. <https://doi.org/10.17795/jjnpp-3530>
- Asuk, A.A., Ugwu N. M. (2018). “Nephroprotective Effect of Vernonia Amygdalina (Bitter Leaf) Extract on Benign Prostatic Hyperplasia in Adult Male Rats”. *International Journal of Biochemistry Research & Review* 22 (4):1-9. <https://doi.org/10.9734/IJBCRR/2018/42910>.
- Awe, O., Odugwu, O., Adebayo, A., & Olapade, O. (2023). Prevalence and clinical presentation of benign prostatic hyperplasia in sub-Saharan Africa: An updated review. *African Journal of Urology*, 29(1), 14–22. <https://doi.org/10.1186/s12301-023-00312-7>
- Awonyemi, O. I., Abegunde, M. S., & Olabiran, T. E. (2020). Analysis of bioactive compounds from *Raphia taedigera* using gas chromatography–mass spectrometry. *Eurasian Chemical Communications*, 2(8), 938–944. <https://doi.org/10.22034/ECC.2020.107898>
- Azizi, A., Mumin, N. H., & Shafqat, N. (2021). Phytochemicals With Anti 5-alpha-reductase Activity: A Prospective For Prostate Cancer Treatment. *F1000Research*, 10, 221. <https://doi.org/10.12688/f1000research.51066.3>
- Azman, N. A. M., Peiró, S., Figueroa, F., & Barrajón-Catalán, E. (2019). Polyphenols: A review of pathway-specific mechanisms of action in cancer. *Nutrients*, 11(10), 2413. <https://doi.org/10.3390/nu11102413>.
- Banakar, P., & Jayaraj, M. (2018). GC–MS analysis of bioactive compounds from ethanolic leaf extract of *Waltheria indica* Linn. and their pharmacological activities. *International Journal of Pharmaceutical Sciences and Research*, 9(5), 2005–2010. [https://doi.org/10.13040/IJPSR.0975-8232.9\(5\).2005-10](https://doi.org/10.13040/IJPSR.0975-8232.9(5).2005-10)
- Banakar, P., & Jayaraj, R. (2018). GC-MS analysis of phytochemicals and their antimicrobial activity from *Andrographis echinoides*. *International Journal of Pharmacognosy and Phytochemical Research*, 10(2), 64–70. <https://doi.org/10.25258/ijpap.v10i2.11375>.
- Baur, J. A., & Sinclair, D. A. (2006). Therapeutic potential of resveratrol: The in vivo evidence. *Nature Reviews Drug Discovery*, 5(6), 493–506. <https://doi.org/10.1038/nrd2060>.
- Bayfield, R. F., & Cole, E. R. (1980). Colorimetric estimation of vitamin A with trichloroacetic acid. In D. B. McCormick & L. D. Wright (Eds.), *Methods in Enzymology*, Vol. 67 (pp. 189–195). Academic Press
- Belakhdar, G., Benjouad, A., & Abdennebi, E. H. (2015). Determination of some bioactive chemical constituents from *Thesium humile* Vahl. *Journal of Materials and Environmental Sciences*, 6(10), 2778–2783.
- Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: The FRAP assay. *Analytical Biochemistry*, 239(1), 70–76. <https://doi.org/10.1006/abio.1996.0292>

- Bhad, P R., Bobde. M.V., Sibi. G. (2021). Chemical constituents and biological activities of *Artocarpus heterophyllus* Lam (Jackfruit): A review. *International Journal of Clinical Microbiology, Biochemistry and Technology*, 4: 005-009. <https://doi.org/10.29328/journal.ijcmbt.1001019>.
- Bouillon, R., Marcocci, C., Carmeliet, G., Bikle, D., White, J. H., Dawson-Hughes, B., ... Lips, P. (2019). Skeletal and extraskeletal actions of vitamin D: Current evidence and outstanding questions. *Endocrine Reviews*, 40(4), 1109–1151. <https://doi.org/10.1210/er.2018-00126>.
- Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, 28(1), 25–30. [https://doi.org/10.1016/S0023-6438\(95\)80008-5](https://doi.org/10.1016/S0023-6438(95)80008-5)
- Breser, M.L., Salazar, F.C., Rivero, V.E., Motrich, R.D. (2017). Immunological mechanisms of underlying chronic pelvic pain and prostate inflammation in chronic pelvic pain syndrome. *Frontier Immunology*, 8:898. <https://doi.org/10.3389/fimmu.2017.00898>.
- Briganti, A., Capitanio, U., Suardi, N., Gallina, A., Salonia, A., Bianchi, M., Tutolo, M., Di Girolamo, V., Guazzoni, G., Rigatti, P., & Montorsi, F. (2009). Benign Prostatic Hyperplasia and Its Aetiologies. *European Urology Supplements*, 8(13), 865-871. <https://doi.org/10.1016/j.eursup.2009.11.002>
- Buck A. C. (1996). Phytotherapy for the prostate. *British journal of urology*, 78(3), 325–336. <https://doi.org/10.1046/j.1464-410x.1996.00104.x>
- Buss, A. D., & Butler, M. S. (Eds.). (2010). *Natural product chemistry for drug discovery* (p. 153). The Royal Society of Chemistry.
- Calabrese, E. J., Iavicoli, I., Calabrese, V., & Hormesis, V. C. (2010). Hormesis and plant biology. *Plant Cell Reports*, 29(10), 1075–1088. <https://doi.org/10.1007/s00299-010-0909-9>
- Canter, P. H., Thomas, H., & Ernst, E. (2005). Bringing medicinal plants into cultivation: opportunities and challenges for biotechnology. *Trends in biotechnology*, 23(4), 180–185. <https://doi.org/10.1016/j.tibtech.2005.02.002>
- Cantillo-Zacarías, J. C., García-Mateos, R., Esparza-Torres, F., Martínez-Damián, T., & Hernández-Ramos, L. (2021). Nutritional and antioxidant quality of Jackfruit (*Artocarpus heterophyllus*). *Current Topics in Agronomic Science*, 1(1), 00-00. <http://dx.doi.org/10.5154/r.ctas.2021.06.17a>
- Cantürk, Z., Kocabıyık, E., Öztürk, N., & İlhan, S. (2017). Evaluation of antioxidant and antiproliferative metabolites of *Penicillium flavigenum* isolated from hypersaline environment: Tuz (Salt) Lake by xCELLigence technology. *Microbiology (Moscow, Russian Federation)*, 86(3), 346–354. <https://doi.org/10.1134/S0026261717030055>
- Carr, A. C., & Maggini, S. (2017). Vitamin C and immune function. *Nutrients*, 9(11), 1211. <https://doi.org/10.3390/nu9111211>.

- Carson, C., & Rittmaster, R. (2003). The role of dihydrotestosterone in benign prostatic hyperplasia. *Urology*, 61(4, Supplement 1), 2–7. [https://doi.org/10.1016/S0090-4295\(02\)02315-2](https://doi.org/10.1016/S0090-4295(02)02315-2)
- Centres for Disease Control and Prevention (CDC) (2003). Trends in aging--United States and worldwide. *MMWR. Morbidity and Mortality Weekly Report*, 52(6), 101–106.
- Chandra, R., Singh, S., Verma, N., & Gupta, A. (2022). Protective role of *Artocarpus heterophyllus* leaf extract in streptozotocin-induced diabetic rats: Antioxidant and antihyperglycemic mechanisms. *Journal of Ethnopharmacology*, 288, 114981. <https://doi.org/10.1016/j.jep.2021.114981>
- Chaves, N., Santiago, A., & Alias, J. C. (2020). Quantification of the antioxidant activity of plant extracts: Analysis of sensitivity and selectivity. *Antioxidants*, 9(1), 76. <https://doi.org/10.3390/antiox9010076>.
- Chedea, V. S., Tomoiagă, L., Macovei, □., & Călinoiu, L. F. (2022). Dietary antioxidants in health and disease. *Antioxidants*, 11(12), 2415. <https://doi.org/10.3390/antiox11122415>.
- Chen, S., Yu, H., Luo, H., Wu, Q., Li, C., & Steinmetz, A. (2023). Sustainable utilization and conservation of medicinal plants: Challenges and strategies under climate change. *Frontiers in Plant Science*, 14, 1156248. <https://doi.org/10.3389/fpls.2023.1156248>
- Chewcharat, A., & Thongprayoon, C. (2020). Potassium disorders: Pathophysiology and management. *World Journal of Nephrology*, 9(2), 36–50. <https://doi.org/10.5527/wjn.v9.i2.36>.
- Chikara, S., Nagaprashantha, L. D., Singhal, J., Horne, D., Awasthi, S., & Singhal, S. S. (2018). Oxidative stress and dietary phytochemicals: Role in cancer chemoprevention and treatment. *Cancer Letters*, 413, 122–134. <https://doi.org/10.1016/j.canlet.2017.10.002>.
- Chinedu, E., Arome, D., & Ameh, F. S. (2013). A New Method for Determining Acute Toxicity in Animal Models. *Toxicology International*, 20(3), 224. <https://doi.org/10.4103/0971-6580.121674>
- Choi, D., Kang, W., & Park, T. (2020). Anti-Allergic and Anti-Inflammatory Effects of Undecane on Mast Cells and Keratinocytes. *Molecules*, 25(7), 1554. <https://doi.org/10.3390/molecules25071554>.
- Chughtai, B., Forde, J. C., Thomas, D. D., Laor, L., Hossack, T., Woo, H. H., Te, A. E., Kaplan, S. A. (2016). Benign prostatic hyperplasia. *Nature Reviews. Disease Primers*, 2, 16031. <https://doi.org/10.1038/nrdp.2016.31>
- Chukwuebuka, I. F., Ifenyinwa, C. C., Okorundu, S. I., Nkechinyere, N. R., & Anekwe, I. I. (2024). Dataset of Non-Edible Calabash Chalk's Bioactive Compounds: A Gas Chromatography-Mass Spectrometry Approach. *Asian Science Bulletin*, 2 (3): 221-228. <https://doi.org/10.3923/asb.2024.221.228>.

- Chung, K. T., Wong, T. Y., Wei, C. I., Huang, Y. W., & Lin, Y. (2001). Tannins and human health: A review. *Critical Reviews in Food Science and Nutrition*, 38(6), 421–464. <https://doi.org/10.1080/10408690191109795>.
- Chu-tse, W., Xue-tao, P., Jun-rong, C., & Hui-hua, X. (1993). A new pharmacological activity of dibutyl phthalate (DBP) on selective elimination of tumor cells from bone marrow. *Leukemia research*, 17(7), 557-560.
- Cieslak, M., Dzik, J. M., & Kaczmarek, K. (2021). Clinical significance of haematological parameters in health and disease. *Blood Reviews*, 48, 100768. <https://doi.org/10.1016/j.blre.2021.100768>.
- Ciosek, Ż., Kot, K., Kosik-Bogacka, D., Łanocha-Arendarczyk, N., & Rotter, I. (2023). The role of magnesium in the human body: A review of current knowledge. *Nutrients*, 15(2), 444. <https://doi.org/10.3390/nu15020444>.
- Crowley, L., Cambuli, F., Aparicio, L., Shibata, M., Robinson, B.D. and Xuan, S. (2020). A single-cell atlas of the mouse and human prostate reveals heterogeneity and conservation of epithelial progenitors. *eLife*, 9. <https://doi.org/10.7554/eLife.59465>.
- Dai, J., & Mumper, R. J. (2010). Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules*, 15(10), 7313–7352. <https://doi.org/10.3390/molecules15107313>.
- De Nunzio, C., Aronson, W., Freedland, S. J., Giovannucci, E., & Parsons, J. K. (2012). The correlation between metabolic syndrome and prostatic diseases. *European Urology*, 61(3), 560–570. <https://doi.org/10.1016/j.eururo.2011.11.013>
- Del Rio, D., Zimetti, F., Caffarra, P., & Brighenti, F. (2020). Biomarkers of oxidative stress in human disease: Current evidence and perspectives. *Critical Reviews in Clinical Laboratory Sciences*, 57(6), 460–478. <https://doi.org/10.1080/10408363.2020.1738939>.
- Deng, X., Zheng, W., Zhan, Q., Deng, Y., Zhou, Y., & Bai, L. (2020). New Lead Discovery of Herbicide Safener for Metolachlor Based on a Scaffold-Hopping Strategy. *Molecules*, 25(21), 4986. <https://doi.org/10.3390/molecules25214986>.
- Dike, C.S., Emejulu, A.A., Chukwudoruo, C.S., Akpaki, M.A., Nsofor, W.N., Edom, C.V. (2023) GC-MS and FTIR Analyses of Bioactive Compounds Present in Ethanol Leaf Extract of *Sida Acuta* from Imo State, Nigeri. *GSC Biological and Pharmaceutical Sciences*, 25, 394-404. <https://doi.org/10.30574/gscbps.2023.25.2.0500>
- Domingue. G.J., & Hellstrom. W.J.G. (1998). Prostatitis. *Clinical Microbiology Review* 11: <https://doi.org/10.1128/cmr.11.4.604>.
- Ekeyi, Y., Uchendu, N. O., Anaduaka, E. G., & Ezeanyika, L. U. S. (2021). Ethanol extract of *Cassia sieberiana* leaves ameliorates deviances associated with benign prostatic hyperplasia in rats. *All Life*, 14(1), 473–483. <https://doi.org/10.1080/26895293.2021.1927857>
- Ekor, M. (2021). Herbal medicines: Opportunities and challenges. *Pharmaceutical Biology*, 59(1), 131–148. <https://doi.org/10.1080/13880209.2021.1888268>

- Elwekeel, A., Hassan, M. H. A., Almutairi, E., AlHammad, M., Alwhbi, F., Abdel-Bakky, M. S., Amin, E., & Mohamed, E. I. A. (2023). Anti-Inflammatory, Anti-Oxidant, GC-MS Profiling and Molecular Docking Analyses of Non-Polar Extracts from Five Salsola Species. *Separations*, 10(2), 72. <https://doi.org/10.3390/separations10020072>
- Elizabeth, K., Rao, M.N.A. 1990. Oxygen radical scavenging activity of curcumin. *Int. J. Pharm.* 58, 237 /240.
- Esomonu, U., Obun, C., Ude, R., Igwe, S., Esomchi, C., & Ogolekwu, S. (2024). Prevalence of benign prostatic hyperplasia and prostate cancer among suburban residents in Southern Nigeria. *African Journal of Urology*, 30(1), 1-7. <https://doi.org/10.1186/s12301-024-00470-x>
- Ezeanyika, L. U., Ejike, C. E., Obidoa, O., & Elom, S. O. (2006). Prostate disorders in an apparently normal Nigerian population 1: Prevalence. *Biokemistri*, 18(2).
- Ezugwu, C.H., Joshua, P.E., Chilaka, F.I., Nwodo, O.F.C., Dasafunjo, K., & Ezugwu M.U (2018) Effect of ethanol extract of *Zapoteca portoricensis* stem on liver, kidney and prostatic biomarkers of testosterone-induced benign prostate hyperplasia (BPH) in male albino rats. *Australian Journal of Basic and Applied Sciences*, 12(10): 19-27. <https://doi.org/10.22587/ajbas.2018.12.10.4>.
- FAO. (2011). *Dietary protein quality evaluation in human nutrition*. Report of an FAO Expert Consultation. FAO Food and Nutrition Paper 92. Rome: FAO.
- FAO/WHO. (2010). *Fats and fatty acids in human nutrition: Report of an expert consultation*. FAO Food and Nutrition Paper 91. Rome: FAO/WHO.
- Farag, S. M., Essa, E. E., Alharbi, S. A., Alfarraj, S., & El-Hassan, G. A. (2021). Agro-waste derived compounds (flax and black seed peels): Toxicological effect against the West Nile virus vector, *Culex pipiens* L. with special reference to GC–MS analysis. *Saudi Journal of Biological Sciences*, 28(9), 5261-5267. <https://doi.org/10.1016/j.sjbs.2021.05.038>.
- Faridha Begum, I., Mohankumar, R., Jeevan, M., & Ramani, K. (2016). GC–MS analysis of bio-active molecules derived from *Paracoccus pantotrophus* FMR19 and the antimicrobial activity against bacterial pathogens and MDROs. *Indian Journal of Microbiology*, 56(4), 426–432. <https://doi.org/10.1007/s12088-016-0609-1>.
- Ferdosi, M. F. H., Khan, I. H., & Javaid, A. (2023). Bioactive components of ethyl acetate extract of *Cassia fistula* flowers. *Journal of Animal & Plant Sciences*, 33(3), 511-517. <https://doi.org/10.36899/JAPS.2023.3.0643>.
- Ferdosi, M. F., Javaid, A., & Khan, I. H. (2022). Phytochemical profile of n-hexane flower extract of *Cassia fistula* L. *Bangladesh Journal of Botany*, 51(2), 393-399. <https://doi.org/10.3329/bjb.v51i2.60438>.
- Ferdosi, M. F., Rahman, M. M., & Sultana, S. (2022). Identification of bioactive compounds in medicinal plant extracts by GC-MS and their antibacterial activity. *Journal of Applied*

- Research on Medicinal and Aromatic Plants*, 29, 100373. <https://doi.org/10.1016/j.jarmap.2022.100373>.
- Fernstrom, J. D. (2013). Large neutral amino acids: Dietary effects on brain neurochemistry and function. *Amino Acids*, 45(3), 419–430. <https://doi.org/10.1007/s00726-012-1330-y>.
- Forstermann, U., & Sessa, W. C. (2012). Nitric oxide synthases: Regulation and function. *European Heart Journal*, 33(7), 829–837. <https://doi.org/10.1093/eurheartj/ehr304>.
- Foster C.S. (2009). Pathology of benign prostatic hyperplasia. *Prostate Supplementation*, 9: 4-14.
- Franco, J. V., Tesolin, P., & Jung, J. H. (2023). Update on the management of benign prostatic hyperplasia and the role of minimally invasive procedures. *Prostate International*, 11(1), 1-7. <https://doi.org/10.1016/j.pnil.2023.01.002>
- Franco, J.V., Turk, T., Jung, J.H., Xiao, Y.T., Iakhno, S. and Tirapegui, F.I. (2019). Pharmacological interventions for treating chronic prostatitis/chronic pelvic pain syndrome. Cochrane Database Systematic Review, 10(10): Cd012552. <https://doi.org/10.1002/14651858.CD012552.pub2>.
- Gacci, M., Corona, G., Vignozzi, L., Salvi, M., Serni, S., De Nunzio, C., Tubaro, A., Oelke, M., Carini, M., & Maggi, M. (2015). Metabolic syndrome and benign prostatic enlargement: a systematic review and meta-analysis. *British Journal of Urology international*, 115(1), 24–31. <https://doi.org/10.1111/bju.12728>
- Gade, S., Rajamanikyam, M., Vadlapudi, V., Nukala, K. M., Aluvala, R., Giddigari, C., Deevi, R. K., Pamineni, P., Barua, N. C., Pandey, R., & Upadhyayula, S. M. (2017). Acetylcholinesterase inhibitory activity of stigmasterol and hexacosanol is responsible for larvicidal and repellent properties of *Chromolaena odorata*. *Biochimica et Biophysica Acta (BBA) - General Subjects*, 1861(3), 541–550. <https://doi.org/10.1016/j.bbagen.2016.11.044>
- Gaetke, L. M., & Chow, C. K. (2003). Copper toxicity, oxidative stress, and antioxidant nutrients. *Toxicology*, 189(1–2), 147–163. [https://doi.org/10.1016/S0300-483X\(03\)00159-8](https://doi.org/10.1016/S0300-483X(03)00159-8).
- Galli, F., Azzi, A., Birringer, M., Cook-Mills, J. M., Eggersdorfer, M., Frank, J., ... Özer, N. K. (2023). Vitamin E: Emerging aspects and new directions. *Free Radical Biology and Medicine*, 198, 81–90. <https://doi.org/10.1016/j.freeradbiomed.2023.03.011>.
- Gann P.H, Hennekens C.H, Longcope C, Verhoek-Oftedahl W, Grodstein F, Stampfer M.J. (1995). A prospective study of plasma hormone levels, nonhormonal factors, and development of benign prostatic hyperplasia. *Prostate*, 26(1):40-9.
- Gao, J., Li, X., Wang, Y., & Sun, H. (2022). Clinical implications of serum proteins in liver disease: Focus on albumin and globulin. *World Journal of Hepatology*, 14(6), 1121–1134. <https://doi.org/10.4254/wjh.v14.i6.1121>.
- García, D., Girardi, N. S., Passone, M. A., Nesci, A. V., & Etcheverry, M. G. (2017). Harmful effects on *Oryzaephilus surinamensis* (L.) and *Tribolium castaneum* by food-grade antioxidants and their formulations in peanut kernel. *Journal of Food Chemistry & Nanotechnology*, 3(3), 86–92. <https://doi.org/10.17756/jfcn.2017-042>.

- Ghosh, A., Dutta, D., Banerjee, S., & Basu, A. (2022). Phytochemicals as potential therapeutic agents in kidney diseases. *Frontiers in Pharmacology*, 13, 940325. <https://doi.org/10.3389/fphar.2022.940325>.
- Goswami, C., Hossain, M. A., Kader, H. A., & Islam, R. (2011). Assessment of physicochemical properties of jackfruits' (*Artocarpus heterophyllus* Lam) pulps. *Journal of Horticulture, Forestry and Biotechnology*, 15(3), 26-31.
- Green, L. C., Wagner, D. A., Glogowski, J., Skipper, P. L., Wishnok, J. S., & Tannenbaum, S. R. (1982). Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. *Analytical biochemistry*, 126(1), 131–138. [https://doi.org/10.1016/0003-2697\(82\)90118-x](https://doi.org/10.1016/0003-2697(82)90118-x)
- Growables, 2020. Jackfruit (*Artocarpus heterophyllus*). Growables.org. Growables Inc., Florida, USA <https://www.growables.org/information/TropicalFruit/jackfruit.htm>
- Gülçin, İ. (2020). Antioxidants and antioxidant methods: An updated overview. *Archives of Toxicology*, 94(3), 651–715. <https://doi.org/10.1007/s00204-020-02689-3>.
- Gullberg, J., Jonsson, P., Nordström, A., Sjöström, M., & Moritz, T. (2004). Design of experiments: An efficient strategy to identify factors influencing extraction and derivatization of *Arabidopsis thaliana* samples in metabolomic studies with gas chromatography/mass spectrometry. *Analytical Biochemistry*, 331(2), 283–295. <https://doi.org/10.1016/j.ab.2004.04.037>.
- Gupta, A., Sharma, S., & Singh, R. (2022). Nutritional modulation of hematopoiesis: Role of micronutrients and bioactive compounds. *Journal of Nutrition and Health Sciences*, 9(2), 45–55. <https://doi.org/10.15761/JNHS.1000358>.
- Gupta, R., Sharma, P., & Kumar, V. (2023). Green synthesis of metallic nanoparticles using fruit and vegetable waste: A sustainable approach for biomedical and environmental applications. *Journal of Cleaner Production*, 412, 137083. <https://doi.org/10.1016/j.jclepro.2023.137083>
- Gutiérrez-Grijalva, E. P., Picos-Salas, M. A., Leyva-López, N., Criollo-Mendoza, M. S., Vazquez-Olivo, G., & Heredia, J. B. (2019). Flavonoids and phenolic acids from *Oregano* (*Origanum vulgare*) with antioxidant and antimicrobial activity. *Food Chemistry*, 139, 154–161. <https://doi.org/10.1016/j.foodchem.2019.03.002>.
- Habermacher, G.M., Chason, J.T., Schaeffer, A.J. (2006). Prostatitis/chronic pelvic pain syndrome. *Annual Review of Medicine*, 57: 195 – 206. <https://doi.org/10.1146/annurev.med.57.011205.135654>.
- Halliwell, B., & Gutteridge, J. M. C. (2015). *Free radicals in biology and medicine* (5th ed.). Oxford University Press.
- Hamazaki, K., Itomura, M., & Hamazaki, T. (2016). Role of cis-vaccenic acid in human health and disease. *Lipids in Health and Disease*, 15, 189. <https://doi.org/10.1186/s12944-016-0375-1>.

- Hamazaki, K., Suzuki, N., Kitamura, K.I., Hattori, A., Nagasawa, T., Itomura, M., and Hamazaki, T. (2016). Is vaccenic acid (18:1t n-7) associated with an increased incidence of hip fracture? An explanation for the calcium paradox. *Prostaglandins Leukot Essent Fatty Acids*; 109: 8–12
- Hamazaki, K., Suzuki, N., Kitamura, K.I., Hattori, A., Nagasawa, T., Itomura, M., and Hamazaki, T. (2016). Is vaccenic acid (18:1t n-7) associated with an increased incidence of hip fracture? An explanation for the calcium paradox. *Prostaglandins Leukot Essent Fatty Acids*; 109: 8–12
- Hasan, M. R., Haque, M. M., Hoque, M. A., Sultana, S., Rahman, M. M., Ali Shaikh, M. A., & Sarker, M. K. U. (2023). Antioxidant activity study and GC-MS profiling of *Camellia sinensis* Linn. *Heliyon*, 10(1), e23514. <https://doi.org/10.1016/j.heliyon.2023.e23514>.
- Hasmila, I., Natsir, H., & Soekamto, N. H. (2019). Phytochemical analysis and antioxidant activity of soursop leaf extract (*Annona muricata* Linn.). *Journal of Physics: Conference Series*, 1341(3), Article 32027. <https://doi.org/10.1088/1742-6596/1341/3/032027>
- Hassan, R., Mensah, A., & Osei, B. (2024). Natural hepatoprotective agents: Advances and prospects in liver disease management. *Biomedicine & Pharmacotherapy*, 168, 115594. <https://doi.org/10.1016/j.biopha.2024.115594>.
- Hatami, S., Mohamadi Sani, A., & Yavarmanesh, M. (2016). Chemical composition and antibacterial activity of organic extra virgin olive oil from Iran. *Nutrition & Food Science*, 46(3), 313–322. <https://doi.org/10.1108/NFS-01-2016-0010>.
- He, H., Luo, H., Xu, H., Qian, B., Zou, X., Zhang, G., Zeng, F., & Zou, J. (2023). Preclinical models and evaluation criteria of prostatitis. *Frontiers in Immunology*, 14, 1183895. <https://doi.org/10.3389/fimmu.2023.1183895>
- Huang, W. Y., Cai, Y. Z., & Zhang, Y. (2010). Natural phenolic compounds from medicinal herbs and dietary plants: Potential use for cancer prevention. *Nutrition and Cancer*, 62(1), 1–20. <https://doi.org/10.1080/01635580903191585>.
- Ihekoronye, A. I., & Ngoddy, P. O. (1985). *Integrated food science and technology for the tropics*. Macmillan Publishers.
- Ijeoma, K. G. (2025). In Silico Molecular Docking Studies of Azadirachta indica Stem Bark Phytochemicals against α -Amylase for Type 2 Diabetes Management. *South Asian Research Journal of Natural Products*, 8(1), 21-31. <https://doi.org/10.9734/sarjnp/2025/v8i1177>.
- Ikuerowo, S. O., Omisanojo, O. A., Bioku, M. J., Ajala, M. O., Nonyelim Mordi, V. P., & Esho, J. O. (2013). Prevalence and characteristics of prostate cancer among participants of a community-based screening in Nigeria using serum prostate specific antigen and digital rectal examination. *The Pan African Medical Journal*, 15, 129. <https://doi.org/10.11604/pamj.2013.15.129.2489>
- Ilic, D., Neuberger, M. M., Djulbegovic, M., & Dahm, P. (2018). Screening for prostate cancer: A Cochrane systematic review. *BMJ Open*, 8(12), e024940.

- Isaacs J.T. (1984). Antagonistic effect of androgen on prostatic cell death. *Prostate*. 1984;5(5):545-57.
- Islam, M. T., Ali, E. S., Uddin, S. J., Shaw, S., Islam, M. A., Ahmed, M. I., ... & Gálvez, J. (2020). Protective roles of flavonoids in nephrotoxicity: Phytotherapy perspectives. *Biomedicine & Pharmacotherapy*, 127, 110181. <https://doi.org/10.1016/j.biopha.2020.110181>
- Iweala, E. E. J., & Ogidigo, J. O. (2015). Prostate specific antigen, antioxidant and hematological parameters in prostatic rats fed *Solanum macrocarpon* L. leaves. *Asian Journal of Biological Sciences*, 8(1), 30-41.
- Izevbigie, E.B. (2003). Discovery of water-soluble anticancer agents (Edotides) from a vegetable found in Benin City, Nigeria. *Experimental Biology and Medicine*. 228: 293-298.
- Jagtap, U., & Bapat, V. (2010). Artocarpus: A review of its traditional uses, phytochemistry and pharmacology. *Journal of Ethnopharmacology*, 129(2), 142-166. <https://doi.org/10.1016/j.jep.2010.03.031>
- Jahan, I., Tona, M. R., Sharmin, S., Sayeed, M. A., Tania, F. Z., Paul, A., Chy, M. N. U., ... & Rahmatullah, M. (2020). GC–MS phytochemical profiling, pharmacological properties, and in silico studies of *Chukrasia velutina* leaves: A novel source for bioactive agents. *Molecules*, 25(15), 3536. <https://doi.org/10.3390/molecules25153536>.
- Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B. B., & Beeregowda, K. N. (2014). Toxicity, mechanism and health effects of some heavy metals. *Interdisciplinary Toxicology*, 7(2), 60–72. <https://doi.org/10.2478/intox-2014-0009>.
- Jasna, T. K., & Khaleel, K. M. (2020). GC-MS analysis of bioactive components of *Kandelia candel* (L.) Druce. *Journal of Advanced Scientific Research*, 11(04 Suppl 9), 193-197.
- Javed, M. R., Salman, M., Tariq, A., Tawab, A., Zahoor, M. K., Naheed, S., Shahid, M., Ijaz, A., & Ali, H. (2022). The Antibacterial and Larvicidal Potential of Bis-(2-Ethylhexyl) Phthalate from *Lactiplantibacillus plantarum*. *Molecules*, 27(21), 7220. <https://doi.org/10.3390/molecules27217220>.
- Johnson-Ajinwo, O. R., & Nyodee, D. G. (2024). Toxicity and Phytochemical Analysis of Five Medicinal Plants. *Archives of Pharmacy and Pharmaceutical Sciences*, 8(2), 029-040.
- Kahokehr, A., Vather, R., Nixon, A., & Hill, A. G. (2013). Non-steroidal anti-inflammatory drugs for lower urinary tract symptoms in benign prostatic hyperplasia: systematic review and meta-analysis of randomized controlled trials. *British Journal of Urology international*, 111(2), 304–311. <https://doi.org/10.1111/j.1464-410X.2012.11559.x>
- Kakkar, S., Bais, S., & Sharma, N. (2014). Therapeutic potential of pyrogallol: A natural polyphenol. *International Journal of Pharmaceutical Sciences Review and Research*, 27(1), 45–52.

- Kareem, K. O., Holdbrooke, S., Aina, O. O., Olaleye, M. T., Ajayi, A. O., Ogunlakin, A. O., ... & Nwozo, S. O. (2024). Phytochemical and GC–MS profiling of ethanol extract of African breadfruit (*Treculia africana* Decne) seeds. *Discover Plants*, 1, 47. <https://doi.org/10.1007/s44372-024-00050-x>
- Kaur, A., Bhardwaj, A., & Garg, R. (2023). Gallic acid: A versatile phytochemical with promising therapeutic applications. *Frontiers in Pharmacology*, 14, 1158243. <https://doi.org/10.3389/fphar.2023.1158243>.
- Kazancıoğlu, R. (2023). Importance of kidney function tests in clinical practice. *Turkish Journal of Nephrology*, 32(1), 1–8. <https://doi.org/10.5152/turkjnephrol.2023.22042>.
- Keke, C. O., Nsofor, W. N., Kumabia, F. K. R., Iloabuchi, G. C., Ejiofor, J. C., & Osuagwu, O. L. (2023). GC–MS and FTIR analysis of ethanol and methanol leaf extract of *Urena lobata* (Caesar weed) for bioactive phytochemical constituents. *Journal of Drug Delivery and Therapeutics*, 13(1), 99–115. <https://doi.org/10.22270/jddt.v13i1.5722>
- Kennedy, D. O. (2016). B vitamins and the brain: Mechanisms, dose and efficacy—A review. *Nutrients*, 8(2), 68. <https://doi.org/10.3390/nu8020068>.
- Kennedy, D. O. (2019). B vitamins and brain development: A review of the current evidence. *Current Opinion in Clinical Nutrition & Metabolic Care*, 22(5), 379–385. <https://doi.org/10.1097/MCO.0000000000000591>.
- Khan, F. U., Ihsan, A. U., Khan, H. U., Jana, R., Wazir, J., Khongorzul, P., Waqar, M., & Zhou, X. (2017). Comprehensive overview of prostatitis. *Biomedicine & Pharmacotherapy*, 94, 1064–1076. <https://doi.org/10.1016/j.biopha.2017.08.016>
- Khan, M.R., Omoloso A.D., Kihara, M. (2003), Antibacterial activity of *Artocarpus heterophyllus*. *Fitoterapia*, 74(2003): 501 - 505.
- Khitan, Z. J., & Agarwal, A. (2021). Hyperkalemia: Etiology, pathophysiology, and management. *Journal of Clinical Medicine*, 10(19), 4490. <https://doi.org/10.3390/jcm10194490>.
- Kim, D., Li, A. A., Gadiparthi, C., & Cholankeril, G. (2023). Bilirubin as a biomarker of liver function and disease progression. *Journal of Hepatology Research*, 74(2), 455–467. <https://doi.org/10.1016/j.jhep.2023.01.021>.
- Kim, S. Y., Lee, H. J., & Kim, K. W. (2013). Aesculetin protects against skin damage. *Archives of Dermatological Research*, 305(5), 349–356. <https://doi.org/10.1007/s00403-013-1334-4>.
- Krieger, J.N., Lee, S.W., Jeon, J., Cheah, P.Y., Liong, M.L., Riley, D.E. (2008). Epidemiology of prostatitis. *International Journal of Antimicrobial Agents*, 31(Suppl 1): S85–90. <https://doi.org/10.1016/j.ijantimicag.2007.08.028>.
- Krieger, J.N., Nyberg, L. Jr., Nickel, J.C. (1999). NIH Consensus definition and classification of prostatitis. *Journal of the American Medical Association*, 282(3): 236–237. <https://doi.org/10.1001/jama.282.3.236>.

- Kristal, A. R., Arnold, K. B., Schenk, J. M., Neuhouser, M. L., Weiss, N., Goodman, P., Antvelink, C. M., Penson, D. F., & Thompson, I. M. (2007). Race/ethnicity, obesity, health related behaviors and the risk of symptomatic benign prostatic hyperplasia: results from the prostate cancer prevention trial. *The Journal of Urology*, 177(4), 1395–1591. <https://doi.org/10.1016/j.juro.2006.11.065>
- Kukko, V., Kaipia, A., Talala, K., Taari, K., Tammela, T. L. J., Auvinen, A., & Murtola, T. J. (2018). Allopurinol and risk of benign prostatic hyperplasia in a Finnish population-based cohort. *Prostate Cancer And Prostatic Diseases*, 21(3), 373–378. <https://doi.org/10.1038/s41391-017-0031-8>
- Kumar, A., Singh, S., & Verma, R. (2022). Phytochemical profiling and antibacterial potential of *Artocarpus heterophyllus* seed extracts against multidrug-resistant pathogens. *Journal of Ethnopharmacology*, 293, 115312. <https://doi.org/10.1016/j.jep.2022.115312>
- Kumar, A., Singh, V., & Sharma, R. (2020). Haematological parameters as indicators of immune status in experimental models. *Journal of Immunology Research*, 2020, 123456. <https://doi.org/10.1155/2020/123456>.
- Kumar, R., Singh, A., & Verma, S. (2022). Histopathological assessment of herbal extract effects on organ function: Correlation with biochemical markers. *Journal of Ethnopharmacology*, 281, 114567. <https://doi.org/10.1016/j.jep.2021.114567>.
- Kumar, S., Narwal, S., Kumar, V., & Prakash, O. (2018). α -Amylase inhibitors from plants: A natural approach to treat diabetes. *Pharmacognosy Reviews*, 15(29), 18–30. https://doi.org/10.4103/phrev.phrev_9_18.
- Kumaresan, S., Senthilkumar, V., Stephen, A., & Balakumar, B. S. (2015). GC-MS analysis and PASS-assisted prediction of biological activity spectra of extract of *Phomopsis* sp. isolated from *Andrographis paniculata*. *World Journal of Pharmaceutical Research*, 4(1), 1035–1053.
- Langan, R. C. (2019). Benign prostatic hyperplasia. *Primary Care: Clinics in Office Practice*, 46(2), 223-232.
- Lee, J. H., & Park, S. H. (2023). Dose-dependent effects of phytochemicals on prostate and hepatic tissues: A histological and biochemical study. *Frontiers in Pharmacology*, 14, 114234. <https://doi.org/10.3389/fphar.2023.114234>.
- Lee, J. H., Jia, Y., Thach, T. T., Han, Y., Kim, B., Wu, C., Kim, Y., Seo, W. D., & Lee, S. J. (2017). Hexacosanol reduces plasma and hepatic cholesterol by activation of AMP-activated protein kinase and suppression of sterol regulatory element-binding protein-2 in HepG2 and C57BL/6J mice. *Nutrition research (New York, N.Y.)*, 43, 89–99. <https://doi.org/10.1016/j.nutres.2017.05.013>.

- Lee, S. H., Choi, C. W., Kim, H. J., & Hwang, S. H. (2015). Phloroglucinol: A marine polyphenol with therapeutic potential. *Marine Drugs*, 13(2), 1056–1075. <https://doi.org/10.3390/md13021056>.
- Li, Y., Zhao, X., Chen, J., & Xu, H. (2021). Natural antioxidants and their hepatoprotective effects: Molecular insights and therapeutic perspectives. *Frontiers in Pharmacology*, 12, 789456. <https://doi.org/10.3389/fphar.2021.789456>.
- Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., Gargiulo, G., Testa, G., Cacciatore, F., Bonaduce, D., & Abete, P. (2018). Oxidative stress, ageing, and diseases. *Clinical Interventions in Ageing*, 13, 757–772. <https://doi.org/10.2147/CIA.S158513>.
- Liu, Z., Zhang, Y., Hou, C., Wang, Y., Jiao, J., & Li, Y. (2015). Phytosterols as 5 α -reductase inhibitors: An update and review. *Current Pharmaceutical Design*, 21(10), 1391–1400. <https://doi.org/10.2174/1381612821666141211095140>
- Lloyd, G. L., Ricke, W. A., & McVary, K. T. (2019). Inflammation, Voiding And BPH Progression. *The Journal of Urology*, 201(5), 868. <https://doi.org/10.1097/JU.0000000000000049>
- Loeb, S., Bjurlin, M. A., Nicholson, J., Tammela, T. L., Penson, D. F., Carter, H. B., & Carroll, P. (2021). Overdiagnosis and overtreatment of prostate cancer. *European Urology*, 80(5), 567–577. <https://doi.org/10.1016/j.eururo.2021.07.004>
- Loeb, S., Kettermann, A., Carter, H. B., Ferrucci, L., Metter, E. J., & Walsh, P. C. (2009). Prostate volume changes over time: results from the Baltimore Longitudinal Study of Aging. *The Journal of Urology*, 182(4), 1458–1462. <https://doi.org/10.1016/j.juro.2009.06.047>
- Loizzo, M. R., Tundis, R., Chandrika, U. G., Abeysekera, A. M., Menichini, F., & Frega, N. G. (2010). Antioxidant and antibacterial activities on foodborne pathogens of *Artocarpus heterophyllus* Lam.(Moraceae) leaves extracts. *Journal of food Science*, 75(5), M291-M295.
- Lorke D. (1983). A new approach to practical acute toxicity testing. *Archives of toxicology*, 54(4), 275–287. <https://doi.org/10.1007/BF01234480>
- Lubran, M.M. (1978) The Measurement of the Serum Total Proteins by the Biuret Method. *Annals of Clinical Laboratory Sciences*, 8, 106-110.
- Maggini, S., Pierre, A., & Calder, P. C. (2018). Immune function and micronutrient requirements change over the life course. *Nutrients*, 10(10), 1531. <https://doi.org/10.3390/nu10101531>.
- Magoha, G. A. (2008). Clinical significance of prostate-specific antigen in black patients with prostatic diseases. *African Journal of Urology*, 14(1), 15–20.
- Malik, C.P and Singh, M.B.(1980) “Plant Enzymology and Histo Enzymology,” Kalyani Publishers, New Delhi, p. 286.
- Maqsood, S., Benjakul, S., Shahidi, F. (2014). Emerging role of phenolic compounds as natural food additives in fish and fish products. *Critical Reviews in Food Science and Nutrition*, 54(12), 1629–1645. <https://doi.org/10.1080/10408398.2011.645203>.

- Marín, T., Contreras, C., & Villalobos, C. (2023). Nitric oxide as a modulator of oxidative stress in health and disease. *Free Radical Biology and Medicine*, 197, 45–58. <https://doi.org/10.1016/j.freeradbiomed.2023.03.011>.
- Maruna, R. F. (1957). Serum sodium determination; critical study on colorimetric determination and method]. *International Journal of Clinical Chemistry*, 2(6), 581–585. [https://doi.org/10.1016/0009-8981\(57\)90064-5](https://doi.org/10.1016/0009-8981(57)90064-5)
- Maserejian, N. N., Wager, C. G., Giovannucci, E. L., Curto, T. M., McVary, K. T., & McKinlay, J. B. (2013). Intake of Caffeinated, Carbonated, or Citrus Beverage Types and Development of Lower Urinary Tract Symptoms in Men and Women. *American Journal of Epidemiology*, 177(12), 1399. <https://doi.org/10.1093/aje/kws411>
- McCance, K. L., & Huether, S. E. (2018). *Study Guide for Pathophysiology-E-Book: The Biological Basis for Disease in Adults and Children*. Elsevier Health Sciences.
- McNeal, J.E. (1984). Anatomy of the prostate and morphogenesis of BPH. *Programme of Clinical Biology Research*, 145: 27–53
- McVary, K. T., Roehrborn, C. G., Avins, A. L., Barry, M. J., Bruskewitz, R. C., Donnell, R. F., ... McConnell, J. D. (2021). American Urological Association Guideline: Management of Benign Prostatic Hyperplasia (BPH). *The Journal of Urology*, 206(4), 806–814. <https://doi.org/10.1097/JU.0000000000002230>
- Mensah, M. L. K., Komlaga, G., Forkuo, A. D., Firempong, C., Anning, A. K., & Dickson, R. A. (2024). Emerging approaches in the study of medicinal plants: From ethnopharmacology to bioinformatics. *Journal of Ethnopharmacology*, 320, 117940. <https://doi.org/10.1016/j.jep.2023.117940>
- Mensor, L. L., Menezes, F. S., Leitão, G. G., Reis, A. S., dos Santos, T. C., Coube, C. S., & Leitão, S. G. (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytotherapy research : PTR*, 15(2), 127–130. <https://doi.org/10.1002/ptr.687>
- Mestas, J., & Hughes, C.C. (2004). Of mice and not men: differences between mouse and human immunology. *Journal of Immunology (Baltimore, Md 1950)* 172(5):2731–2738. <https://doi.org/10.4049/jimmunol.172.5.2731>
- Middleton, E., Kandaswami, C., & Theoharides, T. C. (2000). The effects of plant flavonoids on mammalian cells. *Pharmacological Reviews*, 52(4), 673–751.
- Milaeva, E. R., Berezhnoy, S. V., & Mishchenko, V. P. (2010). Antioxidant properties of hindered phenols and their derivatives. *Russian Chemical Reviews*, 79(3), 213–232. <https://doi.org/10.1070/RC2010v079n03ABEH004078>.
- Moghadamtousi, S. Z., Fadaeinasab, M., Nikzad, S., Mohan, G., Ali, H. M., & Kadir, H. A. (2015). *Annona muricata* (Annonaceae): A Review of Its Traditional Uses, Isolated Acetogenins and

Biological Activities. *International Journal of Molecular Sciences*, 16(7), 15625–15658.
<https://doi.org/10.3390/ijms160715625>

- Mohan, V., Radhakrishnan, S., & Kumar, S. (2024). Hypolipidemic and cardioprotective effects of jackfruit seed extract in diet-induced hyperlipidemic rats. *BMC Complementary Medicine and Therapies*, 24(1), 112. <https://doi.org/10.1186/s12906-024-04112-8>
- Morrison, J., Patel, R., & Singh, D. (2021). Phytochemicals as modulators of cytokine activity: Implications for inflammation and chronic disease. *Journal of Ethnopharmacology*, 276, 114182. <https://doi.org/10.1016/j.jep.2021.114182>
- Morrison, J., Patel, R., & Thomas, S. (2021). *Artocarpin as a promising bioactive flavonoid: Antioxidant, anti-inflammatory, and anticancer perspectives*. *Journal of Ethnopharmacology*, 276, 114–122. <https://doi.org/10.1016/j.jep.2021.114122>
- Morrison, M., Patel, H., & Brown, D. (2021). Flavonoid derivatives from tropical plants as potential anticancer agents: Mechanistic insights and therapeutic prospects. *Journal of Ethnopharmacology*, 276, 114215. <https://doi.org/10.1016/j.jep.2021.114215>
- Moron, M. S., Depierre, J. W., & Mannervik, B. (1979). Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochimica et biophysica acta*, 582(1), 67–78. [https://doi.org/10.1016/0304-4165\(79\)90289-7](https://doi.org/10.1016/0304-4165(79)90289-7)
- Morton, J. F., (1987). Jackfruit (*Artocarpus heterophyllus*). In: *Fruits of warm climates*, Miami, FL, 58-64. https://hort.purdue.edu/newcrop/morton/jackfruit_ars.html#Food%20Uses
- Moura, A. C., Rodrigues, F. M., & Oliveira, P. R. (2022). Immunomodulatory effects of plant-derived bioactive compounds: A review. *Frontiers in Immunology*, 13, 846321. <https://doi.org/10.3389/fimmu.2022.846321>
- Mousavi, S. M., Milajerdi, A., Varkaneh, H. K., Gorjipour, M. M., & Esmailzadeh, A. (2021). Vitamin C supplementation and blood pressure: A systematic review and meta-analysis of randomized controlled trials. *Journal of Human Hypertension*, 35(3), 191–201. <https://doi.org/10.1038/s41371-020-0325-4>.
- Muriithi, N. J., Maina, J. W., & Muchugi, A. (2023). Antioxidant defense systems and their potential in reducing oxidative stress in chronic diseases. *Frontiers in Molecular Biosciences*, 10, 1140037. <https://doi.org/10.3389/fmolb.2023.1140037>.
- Nagai, K., Matsuo, S., & Yoshida, A. (2022). Electrolyte imbalance and acid–base disorders in chronic kidney disease. *Kidney Research and Clinical Practice*, 41(2), 129–141. <https://doi.org/10.23876/j.krcp.21.173>
- Nandiyanto, A. B. D., Oktiani, R., & Ragadhita, R. (2019). How to read and interpret FTIR spectroscopy of organic material. *Indonesian Journal of Science and Technology*, 4(1), 97–118. <https://doi.org/10.17509/ijost.v4i1.15806>.

- National Cancer Institute. (2024). Prostate-specific antigen (PSA) test. <https://www.cancer.gov/types/prostate/psa-fact-sheet>
- Natta, L., Srichairatanakool, S., & Khampitak, T. (2023). Anti-inflammatory and analgesic activities of *Artocarpus heterophyllus* Lam. seed and leaf extracts in experimental animal models. *BMC Complementary Medicine and Therapies*, 23(1), 65. <https://doi.org/10.1186/s12906-023-03911-7>
- Natta, L., Srichana, D., & Wongsap, P. (2023). Bioactive compounds and pharmacological potential of *Artocarpus heterophyllus* Lam.: A comprehensive review. *Plants*, 12(6), 1457. <https://doi.org/10.3390/plants12061457>
- Natta, L., Srichana, T., & Ruchirawat, S. (2023). Antibacterial and antibiofilm properties of jackfruit (*Artocarpus heterophyllus*) extracts against clinical pathogens. *BMC Complementary Medicine and Therapies*, 23(1), 214. <https://doi.org/10.1186/s12906-023-03912-8>
- Nayak, M., Nagarajan, A., & Majeed, M. (2017). Pharmacognostic evaluation of leaf and stem wood extracts of *Artocarpus hirsutus* Lam. *Pharmacognosy Journal*, 9(6), 887-894. <http://dx.doi.org/10.5530/pj.2017.6.139>.
- Nchimbi, H. Y. (2025). *Phytochemistry, pharmacology, and growth-inhibition effects of Euphorbia tirucalli, a medicinal plant in Tanzania's semi-arid areas* (pp. 1–19). SSRN. <https://doi.org/10.2139/ssrn.5087507>
- Negi, J.S., Singh, P., Joshi, G.P., Rawat, M.S., & Bisht, V.K. (2004). Chemical constituents of *Gentianaceae* family and their medicinal significance. *Journal of Scientific and Industrial Research*, 63(7), 563–569.
- Newman, D. J., & Cragg, G. M. (2020). Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *Journal of Natural Products*, 83(3), 770–803. <https://doi.org/10.1021/acs.jnatprod.9b01285>
- Ng, M., & Baradhi, K. (2022). Benign Prostatic Hyperplasia. National Library of Medicine.
- Ng, M, Leslie S.W, Baradhi K.M.(2024) Benign Prostatic Hyperplasia. [Updated 2024 Oct 20]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK558920/>
- Niazi, S. K., Basavarajappa, D. S., Kumaraswamy, S. H., Bepari, A., Hiremath, H., Nagaraja, S. K., Rudrappa, M., Hugar, A., Cordero, M. A. W., & Nayaka, S. (2023). GC-MS Based Characterization, Antibacterial, Antifungal and Anti-Oncogenic Activity of Ethyl Acetate Extract of *Aspergillus niger* Strain AK-6 Isolated from Rhizospheric Soil. *Current Issues in Molecular Biology*, 45(5), 3733-3756. <https://doi.org/10.3390/cimb45050241>.
- Nishiyama, T., Ishikawa, M., Takizawa, I., Anraku, T., Shimura, H., Takahashi, K., & Takahashi, K. (2007). Testosterone-induced oxidative stress and hepatotoxicity in male rats. *Endocrine Journal*, 54(6), 875–881. <https://doi.org/10.1507/endocrj.K07-013>

- Novotny, J. A., Peterson, C. A., & Baer, D. J. (2019). Molybdenum: An essential trace element with beneficial roles. *Advances in Nutrition*, 10(2), 333–344. <https://doi.org/10.1093/advances/nmy108>.
- Nwabueze, A. C., Ezeonu, F. C., Ogbuagu, A. S., & Onyekwelu, O. E. (2022). Nephroprotective and antioxidant effects of medicinal plant extracts in experimental models: A review. *Journal of Ethnopharmacology*, 289, 115050. <https://doi.org/10.1016/j.jep.2021.115050>
- Nygård, L. H., Talala, K., Taari, K., Tammela, T. L. J., Auvinen, A., & Murtola, T. J. (2023). Antidiabetic drugs, glycemic control and risk of benign prostatic hyperplasia. *The Prostate*, 83(3), 246–258. <https://doi.org/10.1002/pros.24456>
- Oberley, R. E., Goss, K. L., Quintar, A. A., Maldonado, C. A., & Snyder, J. M. (2007). Regulation of surfactant protein D in the rodent prostate. *Reproductive Biology and Endocrinology: RB&E*, 5, 42. <https://doi.org/10.1186/1477-7827-5-42>
- Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical biochemistry*, 95(2), 351–358. [https://doi.org/10.1016/0003-2697\(79\)90738-3](https://doi.org/10.1016/0003-2697(79)90738-3)
- Ojewola, R. W., Oridota, E. S., Balogun, O. S., Alabi, T. O., Ajayi, A. I., Olajide, T. A., ... & Ogundare, E. O. (2017). Prevalence of clinical benign prostatic hyperplasia amongst community-dwelling men in a South-Western Nigerian rural setting: A cross-sectional study. *African Journal of Urology*, 23(2), 109-115.
- Oliveira, D.S., Dzinic, S., Bonfil, A.I., Saliganan, A.D., Sheng, S., Bonfil, R.D. (2016). The mouse prostate: a basic anatomical and histological guideline. *Bosnian Journal of Basic Medical Sciences* 16(1):8–13. <https://doi.org/10.17305/bjbms.2016.917>.
- Omar, H. S., El-Beshbishy, H. A., Moussa, Z., Taha, K. F., & Singab, A. N. B. (2011). Antioxidant activity of Artocarpus heterophyllus Lam. (jack fruit) leaves extracts: Remarkable attenuations of hyperglycemia and hyperlipidemia in streptozotocin-diabetic rats. *The Scientific World Journal*, 11, 788–800. <https://doi.org/10.1100/tsw.2011.71>.
- Orwa, C.; Mutua, A.; Kindt, R.; Jamnadass, R.; Anthony, S. (2009). Agroforestry Database: a tree reference and selection guide version 4.0. World Agroforestry Centre, Kenya
- Padma, M., Ganesan, S., Jayaseelan, T., Azhagumadhavan, S., Sasikala, P., Senthilkumar, S., & Mani, P. (2019). Phytochemical screening and GC-MS analysis of bioactive compounds present in ethanolic leaves extract of *Silybum marianum* (L). *Journal of Drug Delivery & Therapeutics*, 9(1): 85-89. <http://dx.doi.org/10.22270/jddt.v9i1.2174>.
- Pandey, K. B., Mishra, N., & Rizvi, S. I. (2017). Plant secondary metabolites in health and disease: Pharmacological relevance and therapeutic potential. *Current Pharmaceutical Design*, 23(27), 4047–4062. <https://doi.org/10.2174/1381612823666170714150042>.

- Parastar, H., Jalali-Heravi, M., & Jalali-Heravi, M. (2020). Application of FTIR spectroscopy coupled with chemometric methods to food and plant analysis. *Food Analytical Methods*, 13(3), 682–694. <https://doi.org/10.1007/s12161-019-01677-w>.
- Parsons, J. K., & Kashefi, C. (2008). Physical activity, benign prostatic hyperplasia, and lower urinary tract symptoms. *European urology*, 53(6), 1228-1235.
- Parsons, J. K., Bergstrom, J., Silberstein, J., & Barrett-Connor, E. (2008). Prevalence and Characteristics of Lower Urinary Tract Symptoms in Men Aged 80 Years and Older. *Urology*, 72(2), 318. <https://doi.org/10.1016/j.urology.2008.03.057>
- Parsons, J. K., Sarma, A. V., McVary, K., & Wei, J. T. (2013). Obesity and benign prostatic hyperplasia: clinical connections, emerging etiological paradigms and future directions. *The Journal of Urology*, 189(1), S102–S106. <https://doi.org/10.1016/j.juro.2012.11.029>
- Partin, A. W., Kavoussi, L. R., Peters, C. A., & Dmochowski, R. R. (2021). *Campbell Walsh Wein Handbook of Urology-E-Book*. Elsevier Health Sciences.
- Patel, N. D., & Parsons, J. K. (2014). Epidemiology and etiology of benign prostatic hyperplasia and bladder outlet obstruction. *Indian Journal of Urology : IJU : Journal of the Urological Society of India*, 30(2), 170. <https://doi.org/10.4103/0970-1591.126900>
- Patel, S., Kaur, G., & Singh, J. (2024). Functional food applications of jackfruit (*Artocarpus heterophyllus*): Nutritional, nutraceutical, and technological perspectives. *Food Research International*, 176, 113278. <https://doi.org/10.1016/j.foodres.2024.113278>
- Pilz, S., Zittermann, A., Trummer, C., Theiler-Schwetz, V., Lerchbaum, E., Keppel, M. H., ... März, W. (2022). Vitamin D testing and treatment: A narrative review of current evidence. *Endocrine Connections*, 11(3), e210465. <https://doi.org/10.1530/EC-21-0465>.
- Pisoschi, A. M., & Pop, A. (2015). The role of antioxidants in the chemistry of oxidative stress: A review. *European Journal of Medicinal Chemistry*, 97, 55–74. <https://doi.org/10.1016/j.ejmech.2015.04.040>.
- Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D., & Bitto, A. (2017). Oxidative stress: Harms and benefits for human health. *Oxidative Medicine and Cellular Longevity*, 2017, 8416763. <https://doi.org/10.1155/2017/8416763>.
- Pradhan, D., Patra, R. C., & Sarangi, A. (2021). Evaluation of antimicrobial and antioxidant activity of jackfruit (*Artocarpus heterophyllus*) latex against multidrug-resistant bacteria. *Microbial Pathogenesis*, 150, 104705. <https://doi.org/10.1016/j.micpath.2020.104705>
- Praskash, O.M., Rajesh, K., Anurag, M. and Rajiv, G. (2009). *Artocarpus heterophyllus* (Jackfruit): An Overview. *Indian Journal of Pharmacognosy*. 3(6): 353-358.

- Prins, G. S. (2023). *Chapter 20: What is the prostate and what are its functions?* In Handbook of Andrology (3rd ed.). Andrology Society. Retrieved from <https://andrologysociety.org/wp-content/uploads/2023/04/Chapter-20-Prins.pdf>
- Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*, 53(10), 4290–4302. <https://doi.org/10.1021/jf0502698>
- Priyadarshani, A. M., Jansz, E.R. Peiris, H. (2007). Studies on the Carotenoids of Jackfruit (*A. heterophyllum* Lam) from Matale and Kurunegale Districts. *Journal of National Science Foundation, Sri Lanka*. 35(4): 259–262.
- Pulido, R., Bravo, L., & Saura-Calixto, F. (2000). Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *Journal of agricultural and food chemistry*, 48(8), 3396–3402. <https://doi.org/10.1021/jf9913458>
- Quintar, A. A., Doll, A., Leimgruber, C., Palmeri, C. M., Roth, F. D., Maccioni, M., & Maldonado, C. A. (2010). Acute inflammation promotes early cellular stimulation of the epithelial and stromal compartments of the rat prostate. *The Prostate*, 70(11), 1153–1165. <https://doi.org/10.1002/pros.21150>
- Quintar, A.A., Roth, F.D., De Paul, A.L., Aoki, A., Maldonado, C.A. (2006). Toll-like receptor 4 in rat prostate: Modulation by testosterone and acute bacterial infection in epithelial and stromal cells. *Biology and Reproduction*, 75(5):664–672.
- Radi, R. (2018). Oxygen radicals, nitric oxide, and peroxynitrite: Redox pathways in molecular medicine. *Proceedings of the National Academy of Sciences*, 115(23), 5839–5848. <https://doi.org/10.1073/pnas.1804932115>.
- Rahman, M. A., Chowdhury, T., & Hasan, M. K. (2023). Antidiabetic and metabolic regulatory activities of *Artocarpus heterophyllum* in *Drosophila melanogaster* models. *Frontiers in Pharmacology*, 14, 1150932. <https://doi.org/10.3389/fphar.2023.1150932>
- Rahman, M. A., Nahar, N., Mian, A. J., & Mosihuzzaman, M. (1999). Variation of carbohydrate composition of two forms of fruit from jack tree (*Artocarpus heterophyllum* L.) with maturity and climatic conditions. *Food Chemistry*, 65(1), 91–97.
- Rahman, M. A., Nahar, T., & Mian, M. Y. (2019). Nutritional and therapeutic potential of jackfruit (*Artocarpus heterophyllum* Lam.): A review. *Food Research International*, 123, 143–158. <https://doi.org/10.1016/j.foodres.2019.04.018>
- Rahman, M. A., Rahman, M. S., Hossain, M. A., & Akhtar, N. (2023). Natural plant products in inflammation-associated carcinogenesis: Potential preventive and therapeutic approaches. *Biomedicine & Pharmacotherapy*, 165, 115064. <https://doi.org/10.1016/j.biopha.2023.115064>

- Rahman, M., Chowdhury, A., & Hossain, M. (2023). Synergistic effects of *Artocarpus heterophyllus* bark extract with antibiotics against resistant bacterial strains. *Frontiers in Pharmacology*, 14, 1187624. <https://doi.org/10.3389/fphar.2023.1187624>
- Ramalakshmi, S., & Muthuchelian, K. (2011). Analysis of bioactive constituents from the leaves of *Mallotus tetracoccus* (roxb.) Kurz, by gas chromatography-mass spectrometry. *International Journal of Pharmaceutical Sciences and Research*, 2(6), 1449.
- Ranasinghe, R. A. S. N., Maduwanthi, S. D. T., & Marapana, R. A. U. J. (2019). Nutritional and health benefits of jackfruit (*Artocarpus heterophyllus* Lam.): a review. *International journal of food science*, 2019(1), 4327183.
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, 26(9–10), 1231–1237. [https://doi.org/10.1016/S0891-5849\(98\)00315-3](https://doi.org/10.1016/S0891-5849(98)00315-3)
- Rencz, F., Kovács, Á., Brodszky, V., Gulácsi, L., Németh, Z., Nagy, G. J., Nagy, J., Buzogány, I., Böszörményi-Nagy, G., Majoros, A., & Nyirády, P. (2015). Cost of illness of medically treated benign prostatic hyperplasia in Hungary. *International urology and nephrology*, 47(8), 1241–1249. <https://doi.org/10.1007/s11255-015-1028-7>
- Reddy, C. K., Haripriya, S., & Kumar, V. (2022). Resistant starch and bioactive compounds in jackfruit (*Artocarpus heterophyllus*) seeds: Potential functional food ingredients. *Food Chemistry*, 387, 132884. <https://doi.org/10.1016/j.foodchem.2022.132884>
- Renugadevi, G., Rameshkumar, R., & Rajasekaran, A. (2021). Identification of dodecanoic acid and its pharmacological activities: A GC-MS-based study. *Natural Product Research*, 35(23), 4946–4950. <https://doi.org/10.1080/14786419.2020.1755910>
- Rice, R.P., Rice, L.W., Tindall, H.D. (1987). Fruits and Vegetable Production in Africa. Longman, Ghana.;189-258.
- Rodríguez-Espinoza, C., López, M. G., & Ramírez-Anguiano, A. (2021). Nutritional composition and pharmacological activities of jackfruit (*Artocarpus heterophyllus*): An updated review. *Food Research International*, 145, 110380. <https://doi.org/10.1016/j.foodres.2021.110380>
- Rodríguez-Espinoza, S., Hernández-Luna, C., García, M. D., & Flores-López, M. L. (2021). Phytochemical antioxidants and their role in chemoprevention: Evidence from tropical fruits. *Antioxidants*, 10(5), 754. <https://doi.org/10.3390/antiox10050754>
- Rodríguez-Espinoza, S., López-Cobo, A., Garrido, M., & Gómez-Caravaca, A. M. (2021). Bioactive compounds, antioxidant activity, and potential health benefits of *Artocarpus heterophyllus* Lam. (jackfruit): A review. *Plants*, 10(12), 2734. <https://doi.org/10.3390/plants10122734>

- Roehrborn, C. G. (2022). Current medical therapies for men with lower urinary tract symptoms and benign prostatic hyperplasia: Achievements and limitations. *European Urology Supplements*, 21(2), 50–58. <https://doi.org/10.1016/j.eursup.2022.02.004>
- Rohrmann, S., Fallin, M. D., Page, W. F., Reed, T., Partin, A. W., Walsh, P. C., & Platz, E. A. (2006). Concordance rates and modifiable risk factors for lower urinary tract symptoms in twins. *Epidemiology (Cambridge, Mass.)*, 17(4), 419–427. <https://doi.org/10.1097/01.ede.0000219723.14476.28>
- Rohrmann, S., Smit, E., Giovannucci, E., & Platz, E. A. (2005). Association between markers of the metabolic syndrome and lower urinary tract symptoms in the Third National Health and Nutrition Examination Survey (NHANES III). *International Journal of Obesity*, 29(3), 310–316. <https://doi.org/10.1038/sj.ijo.0802881>
- Rosiyana, Y., Ginting, B., Saidi, N., Maulana, I., Murniana, M., & Yahya, M. (2024). Cytotoxic activity of dichloromethane extract of soursop leaf (*Annona muricata* L.) against cancer. *Allelopathy Journal*, 62(2). <https://doi.org/10.26651/allelo.j/2024-62-2-1492>.
- Roy, R. N., Laskar, S., & Sen, S. K. (2006). Dibutyl phthalate, the bioactive compound produced by *Streptomyces albidoflavus* 321.2. *Microbiological research*, 161(2), 121–126. <https://doi.org/10.1016/j.micres.2005.06.007>
- Ruch, R. J., Cheng, S. J., & Klaunig, J. E. (1989). Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis*, 10(6), 1003–1008. <https://doi.org/10.1093/carcin/10.6.1003>
- Russo, G. I., Castelli, T., Privitera, S., Fragalà, E., Favilla, V., Reale, G., & Cimino, S. (2022). Metabolic syndrome and benign prostatic hyperplasia: A systematic review and meta-analysis. *Journal of Endocrinological Investigation*, 45(1), 11–22. <https://doi.org/10.1007/s40618-021-01642-8>
- Saha, S., Sadhukhan, P., & Sil, P. C. (2011). Hydroxyquinol: A potential antioxidant and metal chelator. *Toxicology in Vitro*, 25(2), 374–381. <https://doi.org/10.1016/j.tiv.2010.11.018>.
- Sahoo, N., Manchikanti, P., & Dey, S. (2023). Quality control and standardisation of herbal medicines: Current challenges and future prospects. *Phytomedicine*, 109, 154568. <https://doi.org/10.1016/j.phymed.2022.154568>
- Sahoo, S., Jena, L., Rath, S., & Panda, S. (2021). Fourier-transform infrared (FTIR) spectroscopy: A promising tool for rapid screening of plant bioactive compounds. *Journal of Applied Spectroscopy*, 88(5), 1–12. <https://doi.org/10.1007/s10812-021-01016-y>.
- Sandhu, J. S., Bixler, B. R., Dahm, P., Goueli, R., Kirkby, E., Stoffel, J. T., & Wilt, T. J. (2024). Management of Lower Urinary Tract Symptoms Attributed to Benign Prostatic Hyperplasia (BPH): AUA Guideline Amendment 2023. *Journal of Urology*, 211(1), 11–19. <https://doi.org/10.1097/JU.0000000000003698> (Original work published January 1, 2024)

- Saikia, S.N., Goswami, T. and Ali, F. (1997). Evaluation of Pulp and Paper Making Characteristics of Certain Fast-Growing Plants. *Wood Science Technology*, 31, 467- 475.
- Saini, R., Arora, S., Kumar, R., & Sharma, S. (2023). Phytotherapeutic management of benign prostatic hyperplasia: Current status and future prospects. *Frontiers in Pharmacology*, 14, 1174281. <https://doi.org/10.3389/fphar.2023.1174281>
- Salem, S. H., El-Maraghy, S. S., Abdel-Mallek, A. Y., Abdel-Rahman, M. A. A., Hassanein, E. H. M., Al-Bedak, O. A., & Abd El-Aziz, F. E. A. (2022). The antimicrobial, antibiofilm, and wound healing properties of ethyl acetate crude extract of an endophytic fungus *Paecilomyces* sp. (AUMC 15510) in earthworm model. *Scientific Reports*, 12, 19239. <https://doi.org/10.1038/s41598-022-23831-4>.
- Sales-Campos, H., Souza, P. R., Peghini, B. C., da Silva, J. S., & Cardoso, C. R. (2013). An overview of the modulatory effects of oleic acid in health and disease. *Mini-Reviews in Medicinal Chemistry*, 13(2), 201–210. <https://doi.org/10.2174/138955713804805018>.
- Salim, A.A., Chin, Y.W., and Kinghorn, A.D. (2008). Drug Discovery from Plants. In: Ramawat, K.G. and Merillion, J.M. (Eds). *Bioactive Molecules and Medicinal Plants*. Springer, USA, 10-25.
- Samy, R. P., Sethi, G., Chow, V. T., & Stiles, B. G. (2013). Plant-based hydrocarbon esters from *Tragia involucrata* possess antimicrobial and anti-inflammatory activities. *Infectious disorders drug targets*, 13(2), 141–153. <https://doi.org/10.2174/1871526511313020005>.
- Sanda, M. G., Beaty, T. H., Stutzman, R. E., Childs, B., & Walsh, P. C. (1994). Genetic susceptibility of benign prostatic hyperplasia. *The Journal of Urology*, 152(1), 115–119. [https://doi.org/10.1016/s0022-5347\(17\)32831-8](https://doi.org/10.1016/s0022-5347(17)32831-8)
- Santos, F. A., Costa, C. G., & Oliveira, R. M. (2021). Integrating histopathology and biochemical biomarkers in evaluating organ toxicity of natural compounds. *Toxicology Reports*, 8, 1252–1264. <https://doi.org/10.1016/j.toxrep.2021.07.010>.
- Santos, P. L., Matos, J. P. S., Picot, L., Almeida, J. R., Quintans, J. S., & Quintans-Junior, L. J. (2019). Citronellol, a monoterpene alcohol with promising pharmacological activities-A systematic review. *Food and Chemical Toxicology*, 123, 459-469. <https://doi.org/10.1016/j.fct.2018.11.030>
- Sato, M. and Fujiwara, S. (1996). Flavones with Antibacterial Activity Against Carcinogenic Bacteria. *Journal of Ethnopharmacology*, 54(2-3):171- 176
- Szabados, L., & Savouré, A. (2010). Proline: a multifunctional amino acid. *Trends in Plant Science*, 15(2), 89–97. <https://doi.org/10.1016/j.tplants.2009.11.009>
- Saxena, A., Bawa, A.S. and Raju, P.S. (2009). Phytochemical Changes in Fresh-cut Jackfruit (*Artocarpus heterophyllus* L.) Bulbs during Modified Atmosphere Storage. *Food Chemistry* 115(4):1443-1449.

- Schaeffer, E. M., Srinivas, S., Antonarakis, E. S., Armstrong, A. J., Bekelman, J. E., Cheng, H., D'Amico, A. V., Davis, J. W., Desai, N., Dorff, T. B., et al. (2022). NCCN Guidelines insights: Prostate cancer, version 1.2023. *Journal of the National Comprehensive Cancer Network*, 20(2), 129–139. <https://doi.org/10.6004/jnccn.2022.0008>.
- Schenk, J. M., Calip, G. S., Tangen, C. M., Goodman, P., Parsons, J. K., Thompson, I. M., & Kristal, A. R. (2012). Indications for and use of nonsteroidal antiinflammatory drugs and the risk of incident, symptomatic benign prostatic hyperplasia: results from the prostate cancer prevention trial. *American Journal of Epidemiology*, 176(2), 156–163. <https://doi.org/10.1093/aje/kwr524>
- Scientific Reports. (2023). Identification of bioactive phytochemicals with analgesic and anti-inflammatory potential using GC–MS and in silico methods. *Scientific Reports*, 13, 47737. <https://doi.org/10.1038/s41598-023-47737-x>
- Searcy, R. L., Reardon, J. E., & Foreman, J. A. (1967). A new photometric method for serum urea nitrogen determination. *The American journal of medical technology*, 33(1), 15–20.
- Septama, A. W., & Daud, N. N. N. M. (2021). Artocarpanone isolated from *Artocarpus heterophyllus* heartwoods enhances cytotoxic effect of cisplatin against H460 and MCF-7 cell lines. In *IOP Conference Series: Materials Science and Engineering* (Vol. 1011, No. 1, p. 012015). IOP Publishing.
- Sfanos, K. S., & De Marzo, A. M. (2022). Inflammation and prostate carcinogenesis: Pathways and mechanisms. *Nature Reviews Urology*, 19(3), 139–152. <https://doi.org/10.1038/s41585-021-00543-5>.
- Shahidi, F., & Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects – A review. *Journal of Functional Foods*, 18(Part B), 820–897. <https://doi.org/10.1016/j.jff.2015.06.018>.
- Shahidi, F., & Ambigaipalan, P. (2018). Tocopherols and tocotrienols in edible oils and fats: Chemistry, occurrence, and health effects. *Journal of Food Bioactives*, 1, 48–73. <https://doi.org/10.31665/JFB.2018.1126>.
- Sharma, A., Gupta, S., & Chauhan, R. (2017). Herbal drugs for benign prostatic hyperplasia (BPH): Current status and future prospects. *Pharmacognosy Reviews*, 11(22), 150–160. https://doi.org/10.4103/phrev.phrev_43_17
- Sharma, P., Kumar, V., & Tiwari, S. (2022). Antioxidants and red blood cell protection: Implications for health and disease. *Frontiers in Pharmacology*, 13, 836845. <https://doi.org/10.3389/fphar.2022.836845>.

- Shettar, A. K., Sateesh, M. K., Kaliwal, B. B., & Vedamurthy, A. B. (2017). In vitro antidiabetic activities and GC-MS phytochemical analysis of *Ximenia americana* extracts. *South African Journal of Botany*, *111*, 202–211. <http://dx.doi.org/10.1016/j.sajb.2017.03.014>.
- Shoag, J. E., Mittal, S., Hu, J. C., & Barbieri, C. E. (2021). Association of PSA screening intensity with prostate cancer mortality. *Journal of the American Medical Association Network Open*, *4*(3), e213032.
- Sies, H., & Jones, D. P. (2020). Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. *Nature Reviews Molecular Cell Biology*, *21*(7), 363–383. <https://doi.org/10.1038/s41580-020-0230-3>.
- Singh, A., Singh, V., & Verma, P. K. (2021). Pharmacological and therapeutic potential of jackfruit (*Artocarpus heterophyllus*): A review. *Journal of Food Biochemistry*, *45*(3), e13643. <https://doi.org/10.1111/jfbc.13643>
- Sivanesan, I., Ahn, Y. J., & Jeong, B. R. (2023). Plant-derived antioxidants: Progress in clinical trials. *Antioxidants*, *12*(3), 645. <https://doi.org/10.3390/antiox12030645>.
- Skrajnowska, D., & Bobrowska-Korczak, B. (2019). Role of zinc in immune system and anti-cancer defense mechanisms. *Nutrients*, *11*(10), 2273. <https://doi.org/10.3390/nu11102273>.
- Slavin, J. L. (2013). Carbohydrates, dietary fiber, and resistant starch in white vegetables: Links to health outcomes. *Advances in Nutrition*, *4*(3), 351S–355S. <https://doi.org/10.3945/an.112.003491>.
- Smith, E. A., & Macfarlane, G. T. (1997). Formation of phenolic and indolic compounds by anaerobic bacteria in the human large intestine. *Microbial Ecology*, *33*(3), 180–188. <https://doi.org/10.1007/s002489900024>.
- Snow, N. H., & Slack, G. C. (2002). Head-space analysis in modern gas chromatography. *TrAC — Trends in Analytical Chemistry*, *21*(9–10), 608–617. [https://doi.org/10.1016/S0165-9936\(02\)00802-6](https://doi.org/10.1016/S0165-9936(02)00802-6).
- Soni, H., Patel, R., & Chauhan, K. (2024). Inhibition of quorum sensing and biofilm formation by jackfruit (*Artocarpus heterophyllus*) seed extracts in *Pseudomonas aeruginosa*. *Journal of Applied Microbiology*, *136*(5), 1851–1862. <https://doi.org/10.1111/jam.16042>
- Soni, R., Kumari, A., & Gupta, P. (2024). Immunomodulatory potential of plant-derived flavonoids: Emerging insights for therapy. *Frontiers in Pharmacology*, *15*, 1421983. <https://doi.org/10.3389/fphar.2024.1421983>
- Soni, R., Mehta, A., & Kothari, V. (2024). Antibiofilm and quorum-sensing inhibition potential of *Artocarpus heterophyllus* extracts against multidrug-resistant bacteria. *Journal of Applied Microbiology*, *136*(1), 112–123. <https://doi.org/10.1093/jambio/lxad215>
- Stroup, S. P., Palazzi-Churas, K., Kopp, R. P., & Parsons, J. K. (2012). Trends in adverse events of benign prostatic hyperplasia (BPH) in the USA, 1998 to 2008. *British Journal of Urology International*, *109*(1), 84–87. <https://doi.org/10.1111/j.1464-410X.2011.10250.x>.

- Sugimura, Y., Cunha, G.R., Donjacour, A.A., Bigsby, R.M., Brody, J.R.(1986). Whole-mount autoradiography study of DNA synthetic activity during postnatal development and androgen-induced regeneration in the mouse prostate. *Biology and Reproduction*, 34(5):985– 995. <https://doi.org/10.1095/biolreprod34.5.985>.
- Sun, W., & Shahrajabian, M. H. (2023). Therapeutic Potential of Phenolic Compounds in Medicinal Plants—Natural Health Products for Human Health. *Molecules*, 28(4), 1845. <https://doi.org/10.3390/molecules28041845>.
- Suresh, K., Das, S., & Ramesh, R. (2021). Blood cell counts and immune function: Clinical and experimental perspectives. *Clinical Haematology*, 34(2), 89–102. <https://doi.org/10.1016/j.clhe.2021.01.004>
- Swami, S. B., Thakor, N. J., Haldankar, P. M., & Kalse, S. B. (2021). Jackfruit and its many functional components as related to human health: A review. *Comprehensive Reviews in Food Science and Food Safety*, 20(6), 6012–6038. <https://doi.org/10.1111/1541-4337.12838>
- Sweeney, C.J., Mehrotra, S., Sadaria, M.R., Kumar, S., Shortle, N.H., Roman, Y., Sheridan, C., Campbell, R.A., Murray, D.J., Badve, S., Nakshatri, H. (2005). The sesquiterpene lactone parthenolide in combination with docetaxel reduces metastasis and improves survival in a xenograft model of breast cancer. *Molecular Cancer Therapy*; 4(6): 1004.
- Tamma, S. M. L., Kalyanaraman, V. S., Pahwa, S., Dominguez, P., & Modesto, R. R. (2003). The lectin jacalin induces phosphorylation of ERK and JNK in CD4+ T cells. *Journal of Leucocyte Biology*, 73(5), 682-688.
- Tan, Y. S., Lim, Y. Y., & Wong, S. K. (2023). Comparative evaluation of antioxidant activities of different plant parts: A systematic review. *Plants*, 12(4), 876. <https://doi.org/10.3390/plants12040876>
- Tang, Y., Li, H., & Song, Q. (2024). Lemongrass essential oil and its major component citronellol: evaluation of larvicidal activity and acetylcholinesterase inhibition against *Anopheles sinensis*. *Parasitology Research*, 123(9), 315. <https://doi.org/10.1007/s00436-024-08338-3>.
- Taylor, J.L. (1993). Forest Monks and the Nation State. An Anthropological and Historical Study in Northeast Thailand. 218Pp.
- Tesoriere, L. Butera, D; Allegra, M, Fazzari M, and Liurea, M.A. (2005). Distribution of Betalane Pigments in Red Blood Cells after Consumption. Cactus Pear Fruit and Increased Resistance of the Cells to Ex-vivo include Oxidative. *Journal of Agriculture and Food Chemistry*. 53:1266-1270.
- Tonisi, S., Okaiyeto, K., Mabinya, L. V., & Okoh, A. I. (2020). Evaluation of bioactive compounds, free radical scavenging and anticancer activities of bulb extracts of *Boophone disticha* from Eastern Cape Province, South Africa. *Saudi Journal of Biological Sciences*, 27(12), 3559–3569. <https://doi.org/10.1016/j.sjbs.2020.07.028>

- Toora, B.D. and Rajagopal, G. (2002) Measurement of Creatinine by Jaffe's Reaction-Determination of Concentration of Sodium Hydroxide Required for Maximum Color Development in Standard, Urine and Protein Free Filtrate of Serum. *Indian Journal of Experimental Biology*, 40, 352-354.
- Trease, G.E. and Evans, W.C. (1989). Pharmacognosy. 13th ed. Bailliere, Tundal Limited, London. 350Pp
- Trindade, M. B., Lopes, J. L., Soares-Costa, A., Monteiro-Moreira, A. C., Moreira, R. A., Oliva, M. L. V., & Beltramini, L. M. (2006). Structural characterization of novel chitin-binding lectins from the genus *Artocarpus* and their antifungal activity. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics*, 1764(1), 146-152.
- Tripathi, K., Kumar, P., Kumar, R., Saxena, R., Kumar, A., Badoni, H., Goyal, B., & Mirza, A. A. (2023). Efficacy of jackfruit components in prevention and control of human disease: A scoping review. *Journal of Education and Health Promotion*, 12, 361. https://doi.org/10.4103/jehp.jehp_1683_22.
- Uka, E., Eghianrunwa, Q. A., & Akwo, V. D. (2022). GC-MS analysis of bioactive compounds in ethanol leaves extract of *sphenocentrum jollyanum* and their biological activities. *International Journal of Scientific Research in Engineering and Management*, 6(01). 1 – 29. <https://doi.org/10.55041/IJSREM11414>.
- Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T., Mazur, M., & Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *International Journal of Biochemistry & Cell Biology*, 39(1), 44–84. <https://doi.org/10.1016/j.biocel.2006.07.001>
- Vazhacharickal, P. J., Sajeshkumar, N. K., Mathew, J. J., Kuriakose, A. C., Abraham, B., Mathew, R. J., Albin, A. N., Thomson, D., Thomas, R. S., Varghese, N., & Jose, S. (2016). Chemistry and medicinal properties of jackfruit (*Artocarpus heterophyllus*): a review on current status of knowledge. *International Journal of Innovative Research and Review*, 3(2), 83-95.
- Vaz-Tostes, M. G., da Silva, B. P., Pires, V. C., & Martino, H. S. D. (2022). Potassium and human health: Current understanding and future perspectives. *Nutrients*, 14(3), 497. <https://doi.org/10.3390/nu14030497>.
- Vera, R., Morales, J., & Pérez, E. (2021). Platelet physiology and oxidative stress: Impact on hemostasis. *Thrombosis Research*, 199, 85–92. <https://doi.org/10.1016/j.thromres.2021.01.013>.
- Vučković, N., Janović, B., & Stevanović, J. (2022). Role of glutathione in oxidative stress and redox signaling. *Antioxidants*, 11(8), 1620. <https://doi.org/10.3390/antiox11081620>.

- Wafy, K. R. (2025). Investigation of antibacterial compounds from actinomycetes against wastewater-associated bacteria. *Water Science*, 39(1), 371-379. <https://doi.org/10.1080/23570008.2025.2510040>.
- Wang, J., Liu, P., Hu, X., & Zhou, R. (2023). Serum transaminases as biomarkers of hepatocellular injury: Recent advances and clinical applications. *Journal of Clinical and Translational Hepatology*, 11(2), 345–354. <https://doi.org/10.14218/JCTH.2023.00015>.
- Wang, X., Chen, X., Zhang, L., & Xu, H. (2021). Oxidative stress and mitochondrial dysfunction in benign prostatic hyperplasia: A review of mechanisms and therapeutic strategies. *Aging and Disease*, 12(6), 1531–1546. <https://doi.org/10.14336/AD.2021.0427>
- Watanabe, F., & Bito, T. (2018). Vitamin B12 sources and bioavailability. *Experimental Biology and Medicine*, 243(2), 148–158. <https://doi.org/10.1177/1535370217746612>.
- WebMD. (2024). Prostate-specific antigen: PSA test and PSA levels explained. <https://www.webmd.com/prostate-cancer/psa>
- Wei, B. L., Weng, J. R., Chiu, P. H., Hung, C. F., Wang, J. P., & Lin, C. N. (2005). Anti-inflammatory flavonoids from *Artocarpus heterophyllus* and *Artocarpus communis*. *Journal of Agricultural and Food Chemistry*, 53(10), 3867–3871. <https://doi.org/10.1021/jf047873n>.
- Wei, J. T., Calhoun, E., & Jacobsen, S. J. (2005). Urologic diseases in America project: benign prostatic hyperplasia. *The Journal of Urology*, 173(4), 1256–1261. <https://doi.org/10.1097/01.ju.0000155709.37840.fe>
- Winterbourn, C. C., Hawkins, R. E., Brian, M., & Carrell, R. W. (1975). The estimation of red cell superoxide dismutase activity. *The Journal of laboratory and clinical medicine*, 85(2), 337–341.
- Wiseman, E. M., Bar-El Dadon, S., & Reifen, R. (2017). The vicious cycle of vitamin A deficiency: A review. *Critical Reviews in Food Science and Nutrition*, 57(17), 3703–3714. <https://doi.org/10.1080/10408398.2016.1160362>.
- World Health Organization. (2022). *WHO global report on traditional and complementary medicine 2021*. Geneva: World Health Organization. <https://www.who.int/publications/i/item/9789240062345>
- Wu, Z., Zhang, L., & Huang, Y. (2022). Role of liver enzymes in oxidative stress and metabolic dysfunction. *Oxidative Medicine and Cellular Longevity*, 2022, 1123456. <https://doi.org/10.1155/2022/1123456>.
- Xia, J., Yin, H., Guo, C., Guo, D., Yu, L., & He, Y. (2013). Testosterone-induced renal injury and its underlying mechanisms in rats. *American Journal of Physiology–Renal Physiology*, 305(6), F1025–F1035. <https://doi.org/10.1152/ajprenal.00171.2013>
- Xu G, Dai G, Huang Z, Guan Q, Du C, Xu X. The Etiology and Pathogenesis of Benign Prostatic Hyperplasia: The Roles of Sex Hormones and Anatomy. *Research and Reports in Urology*. 2024;16:205-214 <https://doi.org/10.2147/RRU.S477396>

- Yin, L., Hu, C., Wang, H., Xu, D., & Liu, J. (2022). Role of growth factors in the pathogenesis of benign prostatic hyperplasia: Emerging targets for therapy. *Urologic Oncology: Seminars and Original Investigations*, 40(3), 93.e1–93.e10. <https://doi.org/10.1016/j.urolonc.2021.10.010>
- Yuniarti, A., Prasetyo, A., & Handayani, W. (2025). Phytochemical profile and therapeutic potentials of *Artocarpus heterophyllus* Lam.: Insights into antioxidant, anti-inflammatory, antidiabetic, and anticancer activities. *Frontiers in Pharmacology*, 16, 1457329. <https://doi.org/10.3389/fphar.2025.1457329>
- Yuniarti, L., Nurjanah, N., & Hidayat, R. (2025). Phytochemical profile and pharmacological potential of *Artocarpus heterophyllus*: Evidence from in vitro, in vivo, and in silico studies. *Journal of Applied Pharmaceutical Science*, 15(1), 45–56. <https://doi.org/10.7324/JAPS.2025.150107>
- Yuniarti, N., Setiawan, B., & Wibowo, S. (2025). Artocarpin from *Artocarpus heterophyllus*: A promising bioactive compound with anticancer and antioxidant potential. *Journal of Herbal Pharmacotherapy*, 25(2), 112–128. <https://doi.org/10.1080/15228947.2025.1145678>
- Yuniarti, N., Wulandari, D., & Hartati, R. (2025). Anti-inflammatory and anticancer potential of artocarpin isolated from *Artocarpus heterophyllus*: Molecular mechanisms and therapeutic implications. *Phytomedicine Plus*, 5(2), 100321. <https://doi.org/10.1016/j.phyplu.2025.100321>
- Yusuf-Salih, B. O., Lateef, A., & Azeez, L. (2024). Phytochemical profiling of wastes from the processing of shea butter (*Vitellaria paradoxa*) and pharmacological potentials within the paradigm of circular bioeconomy: Emphasizing antioxidant and antidiabetic applications. *Cleaner and Circular Bioeconomy*, 9, 100107. <https://doi.org/10.1016/j.clcb.2024.100107>
- Zhang, A., Sun, H., Yan, G., Han, Y., & Wang, X. (2021). Metabolomics and systems pharmacology: Bridging traditional Chinese medicine and molecular pharmacology. *Pharmacological Research*, 165, 105400. <https://doi.org/10.1016/j.phrs.2021.105400>
- Zhang, N., Xu, W., Yan, Y., Chen, M., Li, H., & Chen, L. (2023). Cembrane diterpenoids: Chemistry and pharmacological activities. *Phytochemistry*, 212, 113703. <https://doi.org/10.1016/j.phytochem.2023.113703>
- Zhang, Q., Li, M., Yang, Y., & Zhao, W. (2024). Flavonoid-rich plant extracts as modulators of hepatic enzymes: Implications for liver disease therapy. *Phytomedicine*, 125, 155050. <https://doi.org/10.1016/j.phymed.2024.155050>
- Zhang, Y., Chen, Q., & Zhao, W. (2023). Phytochemicals with hepatoprotective effects: Mechanisms and clinical perspectives. *Frontiers in Pharmacology*, 14, 1213345. <https://doi.org/10.3389/fphar.2023.1213345>
- Zhao, F., Wang, P., Lucardi, R. D., Su, Z., & Li, S. (2020). Natural Sources and Bioactivities of 2,4-Di-Tert-Butylphenol and Its Analogs. *Toxins*, 12(1), 35. <https://doi.org/10.3390/toxins12010035>

- Zhou, Y., Chen, X., Xu, Z., Wang, J., & Liu, J. (2023). Superoxide dismutase and its role in oxidative stress and human diseases. *Redox Biology*, 59, 102561. <https://doi.org/10.1016/j.redox.2023.102561>.
- Zhou, Y., Li, X., & Chen, W. (2020). Role of antioxidants in red blood cell stability and membrane integrity. *Antioxidants*, 9(12), 1232. <https://doi.org/10.3390/antiox9121232>.
- Zhou, Y., Zheng, J., Li, Y., Xu, D. P., Li, S., Chen, Y. M., & Li, H. B. (2016). Natural polyphenols for prevention and treatment of cancer. *Nutrients*, 8(8), 515. <https://doi.org/10.3390/nu8080515>.
- Zih-Rou, H., Kuo-Chuan, H., & Chih-Yang, C. (2009). Squalene: A natural antioxidant and its potential therapeutic applications. *Journal of Traditional and Complementary Medicine*, 9(2), 150–158. <https://doi.org/10.1016/j.jtcme.2009.05.004>

APPENDICES

Appendix 1 : Tables

Appendix 1.1 Effect of ethanol extracts of plant parts of *A. heterophyllus* on antioxidant parameters

Treatment	Antioxidant parameters			
	MDA	GSH	SOD	CAT ()
Control	2.10 ± 0.36 ^a	12.29 ± 0.36 ^a	34.88 ± 0.24 ^a	3.34 ± 0.15 ^a
Negative control	3.16 ± 0.20 ^b	7.96 ± 0.17 ^b	19.84 ± 1.09 ^b	2.04 ± 0.11 ^b
Fansteride (5 mg)	2.02 ± 0.04 ^a	10.16 ± 0.19 ^{cd}	30.38 ± 1.85 ^{ac}	2.82 ± 0.11 ^{cd}
Leaves (250 mg)	2.15 ± 0.18 ^a	9.79 ± 0.23 ^{bc}	27.76 ± 2.83 ^c	2.58 ± 0.22 ^c
Leaves (500 mg)	2.06 ± 0.08 ^a	10.06 ± 0.29 ^{cd}	29.93 ± 0.21 ^{cd}	2.90 ± 0.14 ^{ac}
Stem (250 mg)	2.09 ± 0.11 ^a	11.88 ± 0.15 ^{abe}	31.95 ± 0.53 ^{ac}	2.92 ± 0.03 ^{ac}
Stem (500 mg)	1.98 ± 0.02 ^a	12.16 ± 0.23 ^{ae}	30.87 ± 0.16 ^{ac}	3.05 ± 0.09 ^{ac}
Pulp (250 mg)	2.22 ± 0.18 ^a	9.12 ± 1.12 ^{bc}	28.36 ± 2.46 ^c	2.67 ± 0.06 ^{ce}
Pulp (500 mg)	1.97 ± 0.02 ^a	10.22 ± 0.11 ^{ce}	29.95 ± 0.71 ^{cd}	3.10 ± 0.06 ^{ade}
Ext. Combination (250 mg)	1.91 ± 0.06 ^a	9.91 ± 1.26 ^{bd}	32.05 ± 1.82 ^{ac}	3.21 ± 0.12 ^{ad}
Ext. Combination (500 mg)	2.00 ± 0.05 ^a	11.29 ± 1.31 ^{adef}	33.22 ± 1.87 ^{ad}	3.16 ± 0.19 ^{ade}

Values are mean ± standard deviation (n = 3). Values with different superscript letters per column are statistically significant (p<0.05).

Appendix 1.2 Liver Protein and Bilirubin Concentrations of rats administered various doses of ethanol extract of *A.heterophyllus*

Treatment	TOTAL BILIRUBIN	DIRECT BILIRUBIN	TOTAL PROTEIN	GLOBULIN	ALBUMIN
Control	16.06 ± 0.54 ^a	11.76 ± 0.33 ^a	10.00 ± 0.37 ^a	4.19 ± 0.15 ^{ae}	5.81 ± 0.35 ^a
Negative control	24.40 ± 0.93 ^b	17.21 ± 0.60 ^b	5.29 ± 0.85 ^b	1.88 ± 0.19 ^b	3.76 ± 0.54 ^b
Fansteride (5 mg)	17.63 ± 1.42 ^{ac}	13.28 ± 0.93 ^{ac}	8.74 ± 0.21 ^c	4.42 ± 0.40 ^a	4.32 ± 0.20 ^b
Leaves (250 mg)	18.53 ± 0.70 ^c	14.79 ± 0.52 ^c	7.57 ± 0.27 ^d	3.34 ± 0.20 ^c	4.23 ± 0.07 ^b
Leaves (500 mg)	17.22 ± 0.34 ^{ac}	14.06 ± 0.60 ^c	7.57 ± 0.39 ^d	2.59 ± 0.38 ^d	4.98 ± 0.65 ^{ac}
Stem (250 mg)	16.11 ± 0.24 ^a	13.57 ± 0.27 ^{ac}	8.30 ± 0.19 ^{cd}	3.01 ± 0.09 ^{cd}	5.29 ± 0.24 ^c
Stem (500 mg)	15.31 ± 9.32 ^a	12.54 ± 0.52 ^a	9.39 ± 0.16 ^c	4.20 ± 0.17 ^{ae}	5.19 ± 0.05 ^c
Pulp (250 mg)	18.07 ± 0.29 ^{ac}	13.90 ± 0.55 ^{ac}	6.65 ± 1.13 ^{bd}	2.72 ± 0.73 ^{cd}	3.93 ± 0.40 ^b
Pulp (500 mg)	17.25 ± 0.37 ^{ac}	13.48 ± 0.31 ^{ac}	7.54 ± 1.39 ^{bcd}	2.98 ± 0.51 ^{cd}	4.56 ± 0.88 ^{bc}
Ext. Combination (250 mg)	17.11 ± 0.16 ^{ac}	13.94 ± 0.26 ^{ac}	7.31 ± 0.50 ^{bd}	3.24 ± 0.62 ^{cd}	4.06 ± 0.53 ^b

Ext. Combination (500 mg)	16.50 ± 0.16 ^a	13.03 ± 0.09 ^a	8.66 ± 0.71 ^{cd}	3.77 ± 0.09 ^{ce}	4.88 ± 0.62 ^{bc}
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Values are mean ± standard deviation (n = 3). Values with different superscript letters per column are statistically significant (p<0.05).

Appendix 1.3 Results showing the effect of the plant extract on the liver enzymes in the experimental rats.

Treatment	Liver Enzymes			
	ALT	AST	ALP	LDH
Control	15.14 ± 0.30 ^a	13.15 ± 1.05 ^a	138.60 ± 6.97 ^a	136.40 ± 4.35 ^a
Negative control	22.67 ± 0.62 ^b	23.97 ± 2.28 ^b	179.50 ± 6.96 ^b	132.80 ± 3.06 ^{ab}
Fansteride (5 mg)	18.60 ± 1.05 ^c	16.90 ± 1.43 ^c	152.70 ± 3.78 ^c	128.90 ± 1.27 ^{ab}
Leaves (250 mg)	18.43 ± 0.92 ^{cd}	17.08 ± 0.76 ^c	158.40 ± 2.86 ^c	124.60 ± 4.93 ^b
Leaves (500 mg)	17.85 ± 0.84 ^{cd}	17.20 ± 0.48 ^c	147.10 ± 4.19 ^{ac}	163.20 ± 7.16 ^c
Stem (250 mg)	16.51 ± 0.47 ^{ad}	14.83 ± 0.80 ^{ac}	134.30 ± 3.43 ^a	133.30 ± 3.91 ^{ab}
Stem (500 mg)	15.16 ± 0.22 ^a	12.88 ± 0.15 ^a	129.30 ± 0.94 ^a	159.40 ± 0.76 ^c
Pulp (250 mg)	18.17 ± 0.53 ^{cd}	17.77 ± 0.89 ^c	143.10 ± 2.61 ^{ac}	136.60 ± 4.64 ^{ab}
Pulp (500 mg)	17.81 ± 0.26 ^{cd}	17.68 ± 0.32 ^c	140.50 ± 0.76 ^a	136.50 ± 1.78 ^{ab}
Ext. Combination (250 mg)	16.34 ± 0.63 ^{ad}	16.02 ± 0.25 ^{ac}	140.70 ± 2.63 ^a	137.10 ± 2.48 ^{ab}
Ext. Combination (500 mg)	14.50 ± 0.37 ^a	13.90 ± 0.54 ^a	135.90 ± 1.28 ^a	138.10 ± 2.63 ^{ab}

Values are mean $\pm$ standard deviation (n = 3). Values with different superscript letters per column are statistically significant (p<0.05).

Appendix 1.4 Results showing the effect of the plant extract on the kidney enzymes in the experimental rats.

Treatment	Urea		Creatinine		Sodium		Potassium		Bicarbonate		Chloride	
Control	18.11 0.26 ^a	±	4.60 0.25 ^{ac}	±	169.00 3.66 ^a	±	5.33 0.10 ^{ab}	±	20.50 ± 2.58 ^a		57.89 2.10 ^a	±
Negative control	30.43 1.98 ^b	±	7.27 1.01 ^b	±	187.20 5.65 ^b	±	5.12 0.18 ^{ab}	±	14.34 ± 1.17 ^b		81.77 3.18 ^b	±
Fansteride (5 mg)	19.39 1.04 ^a	±	4.49 0.14 ^a	±	165.40 2.61 ^a	±	5.09 0.52 ^{ab}	±	17.66 ± 0.69 ^c		61.64 1.32 ^a	±
Leaves (250 mg)	25.21 0.31 ^c	±	5.10 0.24 ^c	±	170.60 3.68 ^a	±	5.06 0.08 ^{ab}	±	17.48 ± 0.14 ^c		62.92 9.39 ^{ac}	±
Leaves (500 mg)	23.69 0.37 ^{cd}	±	4.62 0.30 ^{ac}	±	170.80 2.48 ^a	±	4.98 0.15 ^{ab}	±	17.58 ± 0.54 ^c		67.15 1.45 ^c	±
Stem (250 mg)	21.82 0.76 ^d	±	4.66 0.17 ^{ac}	±	175.80 4.36 ^a	±	5.19 0.04 ^{ab}	±	18.13 ± 0.31 ^{ac}		67.52 2.07 ^c	±
Stem (500 mg)	23.77 0.26 ^{cd}	±	5.34 0.28 ^c	±	171.90 2.85 ^a	±	5.60 0.35 ^{ab}	±	16.86 ± 0.36 ^d		59.25 2.95 ^a	±
Pulp (250 mg)	25.52 3.15 ^{cd}	±	5.19 0.24 ^c	±	168.40 1.86 ^a	±	4.93 0.31 ^a	±	16.94 ± 0.31 ^d		66.79 0.58 ^c	±
Pulp (500 mg)	22.47 0.45 ^d	±	5.04 0.17 ^{ac}	±	171.50 3.71 ^a	±	5.25 0.12 ^{ab}	±	17.74 ± 0.17 ^c		68.20 7.30 ^{ac}	±
Ext. Combination (250 mg)	21.17 0.38 ^d	±	4.72 0.21 ^{ac}	±	172.60 4.24 ^a	±	5.20 0.23 ^{ab}	±	18.90 ± 0.14 ^{ac}		61.74 2.30 ^{ac}	±
Ext. Combination (500 mg)	25.05 3.94 ^{cd}	±	6.01 0.62 ^d	±	171.10 2.66 ^a	±	5.80 0.60 ^b	±	20.71 ± 0.17 ^a		59.45 0.89 ^a	±

Values are mean ± standard deviation (n = 3). Values with different superscript letters per column are statistically significant (p<0.05).

Appendix 1.5. Effect of the plant extracts on the white blood cells of the experimental animals

Treatment	WBC	LYM	MON
Control	99.90 $\pm$ 0.00 ^a	27.67 $\pm$ 0.81 ^a	8.63 $\pm$ 0.15 ^a
Negative control	99.90 $\pm$ 0.00 ^a	54.30 $\pm$ 3.52 ^b	7.67 $\pm$ 0.68 ^a
Fansteride (5 mg)	99.90 $\pm$ 0.00 ^a	45.87 $\pm$ 1.36 ^c	19.33 $\pm$ 3.18 ^b
Leaves (250 mg)	99.90 $\pm$ 0.00 ^a	14.50 $\pm$ 3.93 ^d	7.43 $\pm$ 0.47 ^a
Leaves (500 mg)	99.90 $\pm$ 0.00 ^a	48.30 $\pm$ 1.73 ^c	7.83 $\pm$ 0.06 ^a
Stem (250 mg)	99.90 $\pm$ 0.00 ^a	45.30 $\pm$ 2.52 ^c	18.63 $\pm$ 3.72 ^b
Stem (500 mg)	99.90 $\pm$ 0.00 ^a	20.63 $\pm$ 2.57 ^e	7.30 $\pm$ 0.96 ^a
Pulp (250 mg)	99.90 $\pm$ 0.00 ^a	45.20 $\pm$ 2.10 ^c	12.23 $\pm$ 5.29 ^b
Pulp (500 mg)	98.33 $\pm$ 2.71 ^{ab}	21.93 $\pm$ 1.55 ^e	15.43 $\pm$ 4.55 ^b
Ext. Combination (250 mg)	94.60 $\pm$ 4.69 ^b	37.97 $\pm$ 6.25 ^f	11.90 $\pm$ 2.14 ^b
Ext. Combination (500 mg)	99.10 $\pm$ 1.06 ^{ab}	44.67 $\pm$ 5.24 ^{cf}	14.33 $\pm$ 3.03 ^b

Values are mean $\pm$ standard deviation (n = 3). Values with different superscript letters per column are statistically significant (p<0.05).

Appendix 1.6 Effect of the plant extracts on the full blood count of the experimental animals

Treatment	HB	RBC	MCV	MCH	MCHC	PLT
Control	14.23 $\pm$ 1.05 ^a	11.16 $\pm$ 0.97 ^{ab}	36.20 $\pm$ 1.00 ^a	12.90 $\pm$ 2.10 ^a	35.67 $\pm$ 5.75 ^a	9353.00 $\pm$ 585.10 ^a
Negative control	14.10 $\pm$ 0.56 ^a	12.46 $\pm$ 0.44 ^{ab}	36.27 $\pm$ 1.19 ^a	11.30 $\pm$ 0.10 ^a	31.30 $\pm$ 0.70 ^a	9665.00 $\pm$ 311.30 ^a
Fansteride (5 mg)	15.27 $\pm$ 1.01 ^a	12.82 $\pm$ 0.32 ^{ab}	37.03 $\pm$ 0.55 ^a	11.93 $\pm$ 0.90 ^a	32.27 $\pm$ 1.97 ^a	9672.00 $\pm$ 106.30 ^a
Leaves (250 mg)	15.23 $\pm$ 0.12 ^a	11.64 $\pm$ 1.36 ^{ab}	35.83 $\pm$ 0.81 ^a	13.23 $\pm$ 1.70 ^a	37.07 $\pm$ 5.05 ^a	8979.00 $\pm$ 1513.00 ^a
Leaves (500 mg)	15.80 $\pm$ 2.15 ^a	11.00 $\pm$ 2.00 ^b	35.50 $\pm$ 0.82 ^a	12.27 $\pm$ 1.70 ^a	35.50 $\pm$ 4.28 ^a	8806.00 $\pm$ 1811.00 ^a
Stem (250 mg)	17.63 $\pm$ 1.11 ^a	12.62 $\pm$ 0.53 ^{ab}	36.00 $\pm$ 0.66 ^a	13.97 $\pm$ 0.25 ^a	38.97 $\pm$ 0.60 ^a	9912.00 $\pm$ 108.80 ^a
Stem (500 mg)	17.77 $\pm$ 2.58 ^a	11.96 $\pm$ 0.49 ^{ab}	35.90 $\pm$ 0.76 ^a	14.80 $\pm$ 1.99 ^a	41.33 $\pm$ 4.92 ^a	9933.00 $\pm$ 63.02 ^a
Pulp (250 mg)	17.53 $\pm$ 0.93 ^a	13.25 $\pm$ 0.25 ^{ab}	36.97 $\pm$ 0.86 ^a	13.20 $\pm$ 0.44 ^a	35.80 $\pm$ 0.50 ^a	9895.00 $\pm$ 135.90 ^a
Pulp (500 mg)	18.30 $\pm$ 2.39 ^a	13.02 $\pm$ 0.19 ^{ab}	36.30 $\pm$ 0.82 ^a	13.37 $\pm$ 2.20 ^a	38.10 $\pm$ 4.57 ^a	9857.00 $\pm$ 129.10 ^a
Ext. Combination (250 mg)	17.10 $\pm$ 3.48 ^a	12.98 $\pm$ 0.73 ^{ab}	35.03 $\pm$ 2.02 ^a	12.43 $\pm$ 0.45 ^a	35.40 $\pm$ 4.44 ^a	9765.00 $\pm$ 229.20 ^a
Ext. Combination (500 mg)	17.40 $\pm$ 4.17 ^a	14.80 $\pm$ 3.20 ^b	36.50 $\pm$ 1.23 ^a	11.70 $\pm$ 0.69 ^a	32.13 $\pm$ 2.57 ^a	9708.00 $\pm$ 47.17 ^a

Values are mean $\pm$ standard deviation (n = 3). Values with different superscript letters per column are statistically significant (p<0.05).

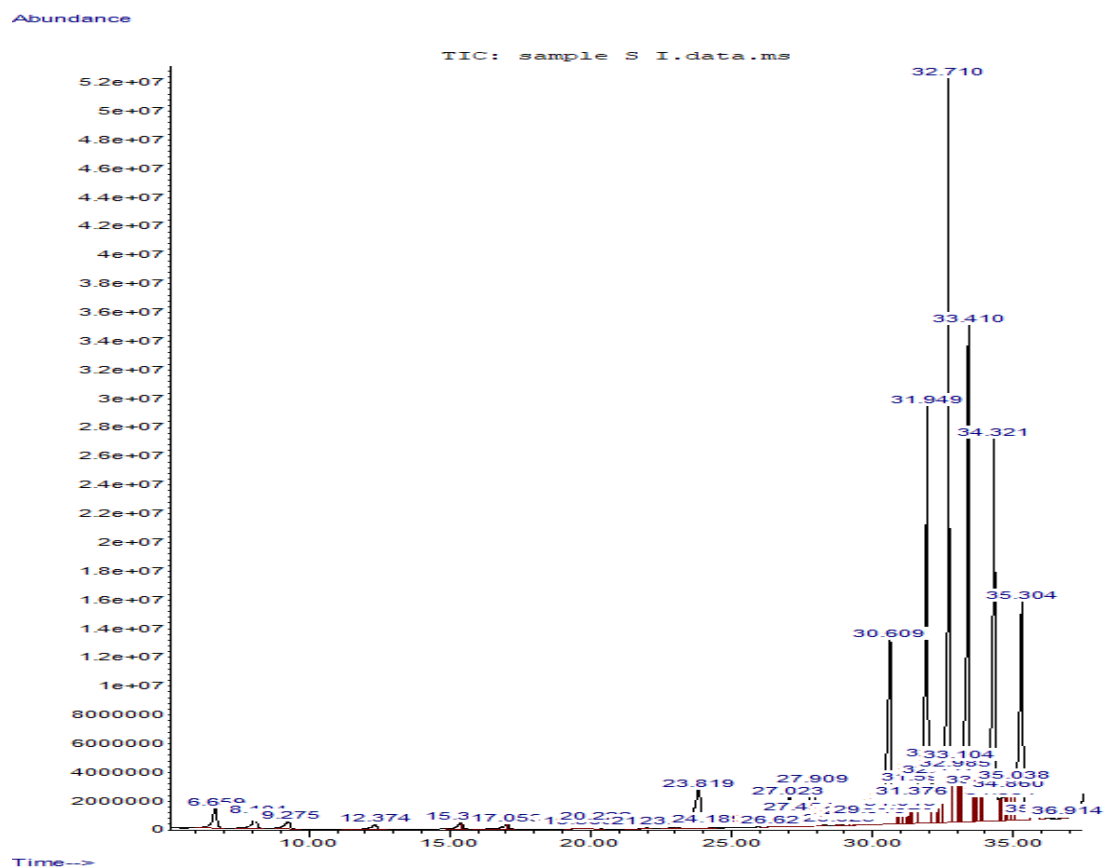
Appendix 1.7. Serum Prostate Marker Levels of rats administered various doses of ethanol extract of *A.heterophyllus*

Treatment	Serum Prostate Marker			
	Testosterone	PSA	TNF	Interleukin
Control	0.54 ± 0.08 ^a	1.37 ± 0.08 ^a	16.23 ± 0.35 ^a	53.20 ± 1.78 ^a
Negative control	3.44 ± 0.19 ^b	3.10 ± 0.03 ^b	25.22 ± 0.89 ^b	85.30 ± 0.39 ^b
Fansteride (5 mg)	1.34 ± 0.18 ^{ce}	1.50 ± 0.11 ^a	17.64 ± 0.11 ^{acd}	61.75 ± 2.35 ^{acd}
Leaves (250 mg)	1.80 ± 0.02 ^{df}	2.19 ± 0.16 ^{cd}	19.73 ± 0.94 ^{de}	72.92 ± 1.87 ^c
Leaves (500 mg)	2.18 ± 0.12 ^d	2.03 ± 0.13 ^{cd}	20.24 ± 0.95 ^e	67.15 ± 2.09 ^{acd}
Stem (250 mg)	1.90 ± 0.24 ^{df}	1.74 ± 0.43 ^{abc}	19.67 ± 0.32 ^{de}	62.50 ± 2.35 ^{acd}
Stem (500 mg)	0.94 ± 0.11 ^{ac}	1.53 ± 0.07 ^{ab}	19.17 ± 0.32 ^{de}	60.25 ± 2.87 ^{ad}
Pulp (250 mg)	1.91 ± 0.19 ^{dg}	2.29 ± 0.07 ^d	20.26 ± 1.81 ^{de}	75.38 ± 4.34 ^c
Pulp (500 mg)	1.54 ± 0.17 ^{cfg}	2.11 ± 0.14 ^{cde}	20.01 ± 0.63 ^{de}	66.24 ± 2.33 ^{acd}
Ext. Combination (250 mg)	1.17 ± 0.10 ^c	1.68 ± 0.21 ^{abef}	18.80 ± 0.31 ^{cde}	63.89 ± 1.69 ^{acd}
Ext. Combination (500 mg)	0.99 ± 0.27 ^{ac}	1.48 ± 0.04 ^{abf}	17.25 ± 0.31 ^{ac}	59.02 ± 1.27 ^{ad}

Values are mean ± standard deviation (n = 3). Values with different superscript letters per column are statistically significant (p<0.05).

APPENDIX 2: Graphs

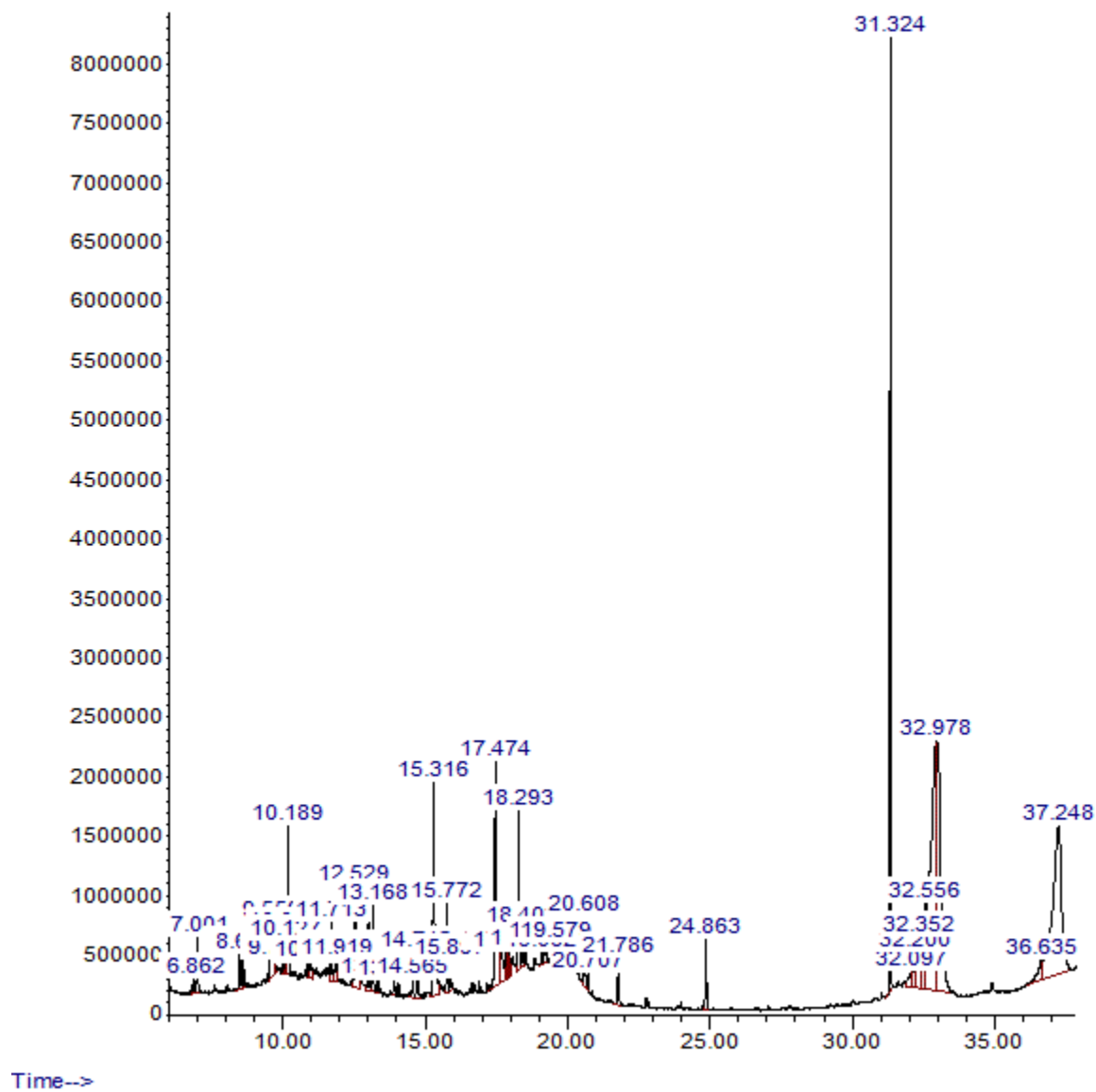
Appendix 2.1 GC-MS Chromatogram of ethanol stem bark extract of *A. heterophyllus*



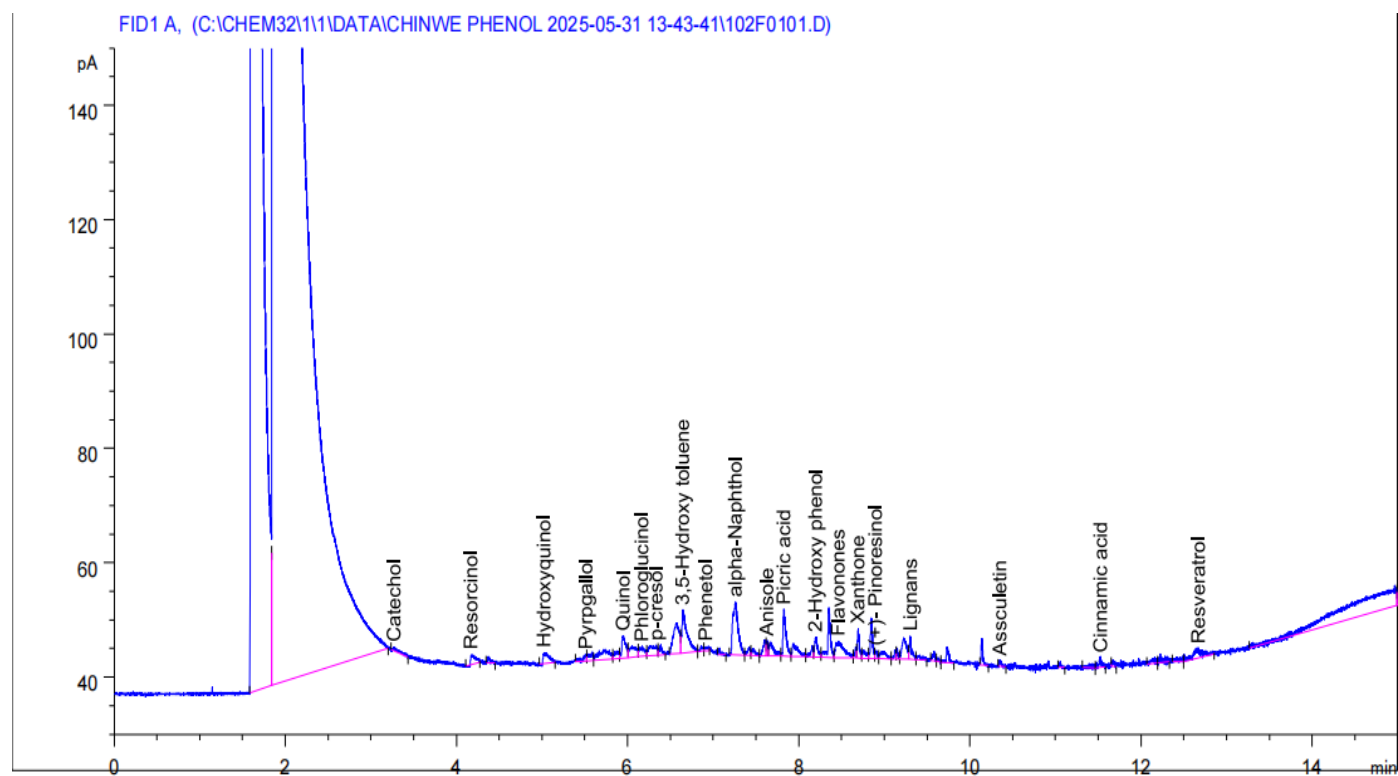
Appendix 2.2 GC-MS Chromatogram ethanol leaf extract of *A. heterophyllus*

Abundance

TIC: L.I.data.ms

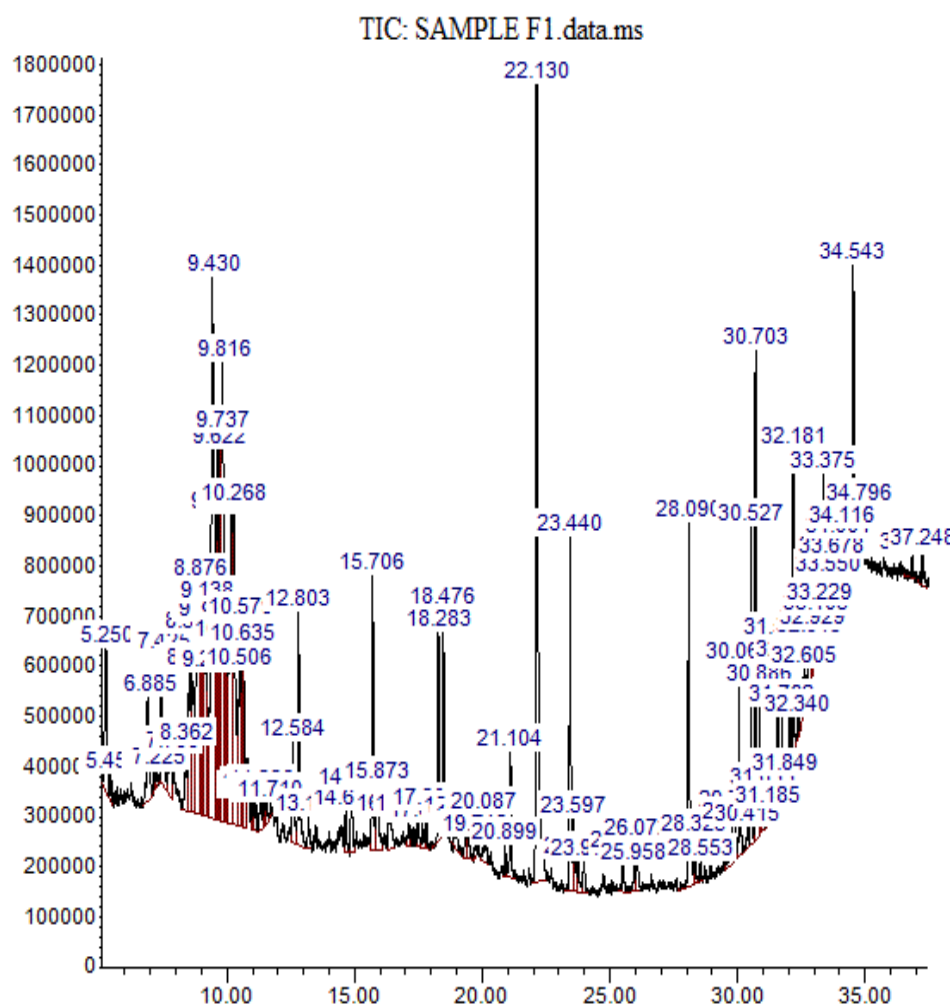


Appendix 2.3 GC-MS Chromatogram ethanol pulp extract of *A. heterophyllus*

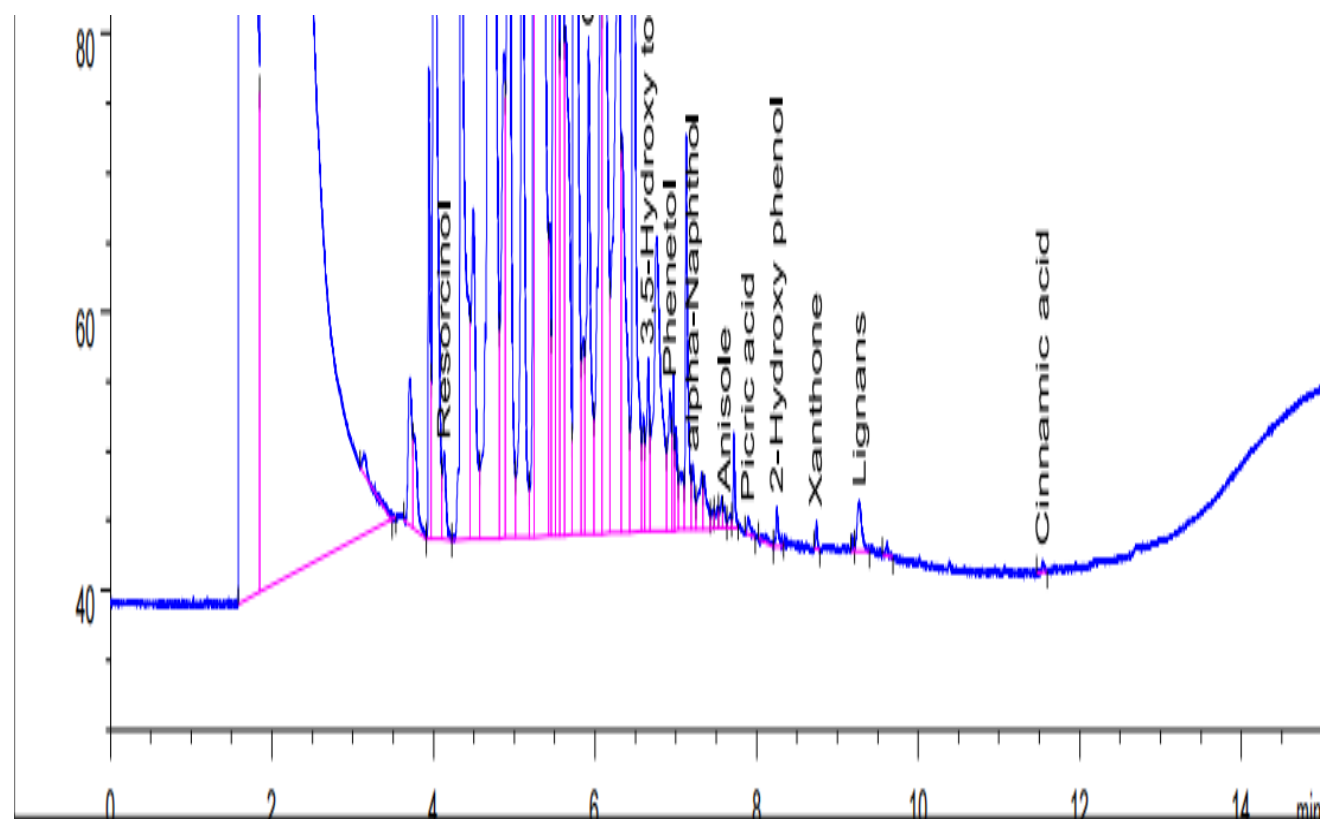


Appendix 2.4 GC-FID Chromatogram For Ethanolic Stem Bark Extract Of *A. Heterophyllus*

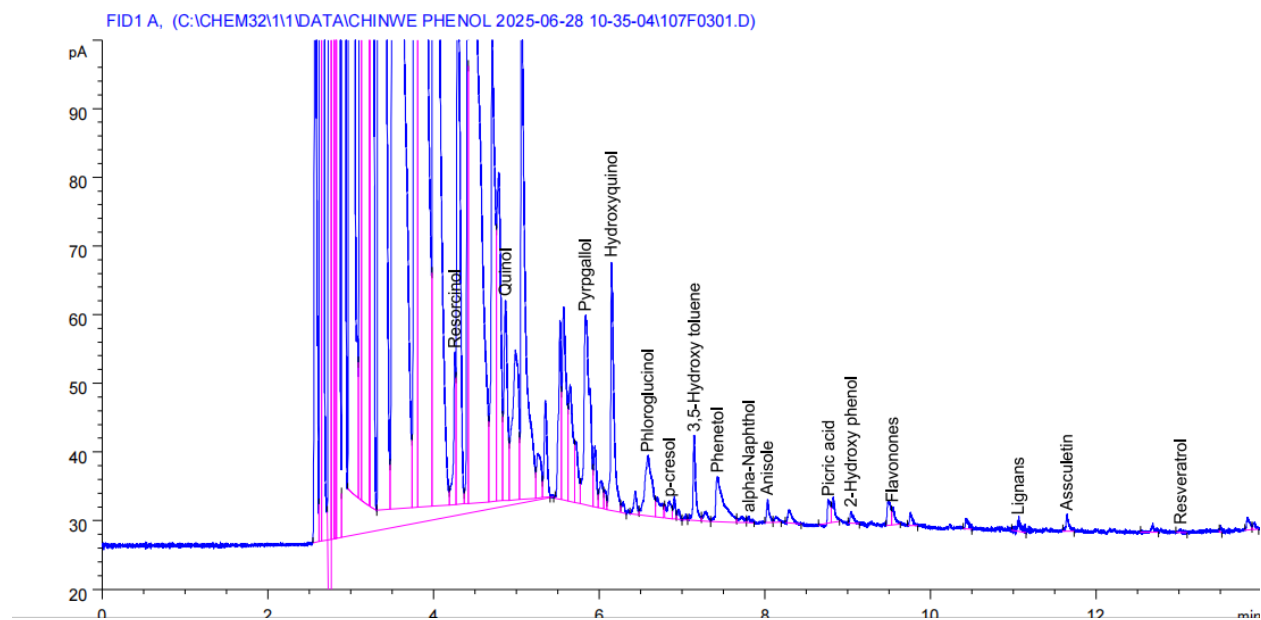
Abundance



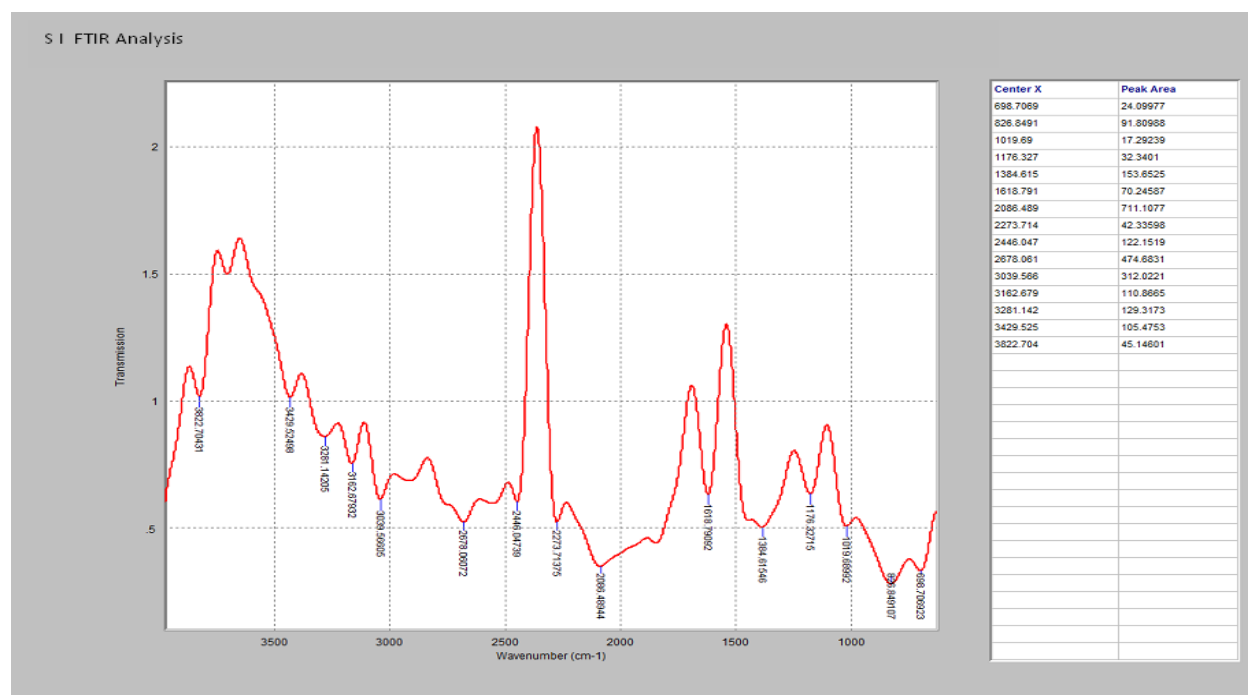
Appendix 2.5 GC-FID Chromatogram For Ethanolic leaf Extract Of *A. Heterophyllus*



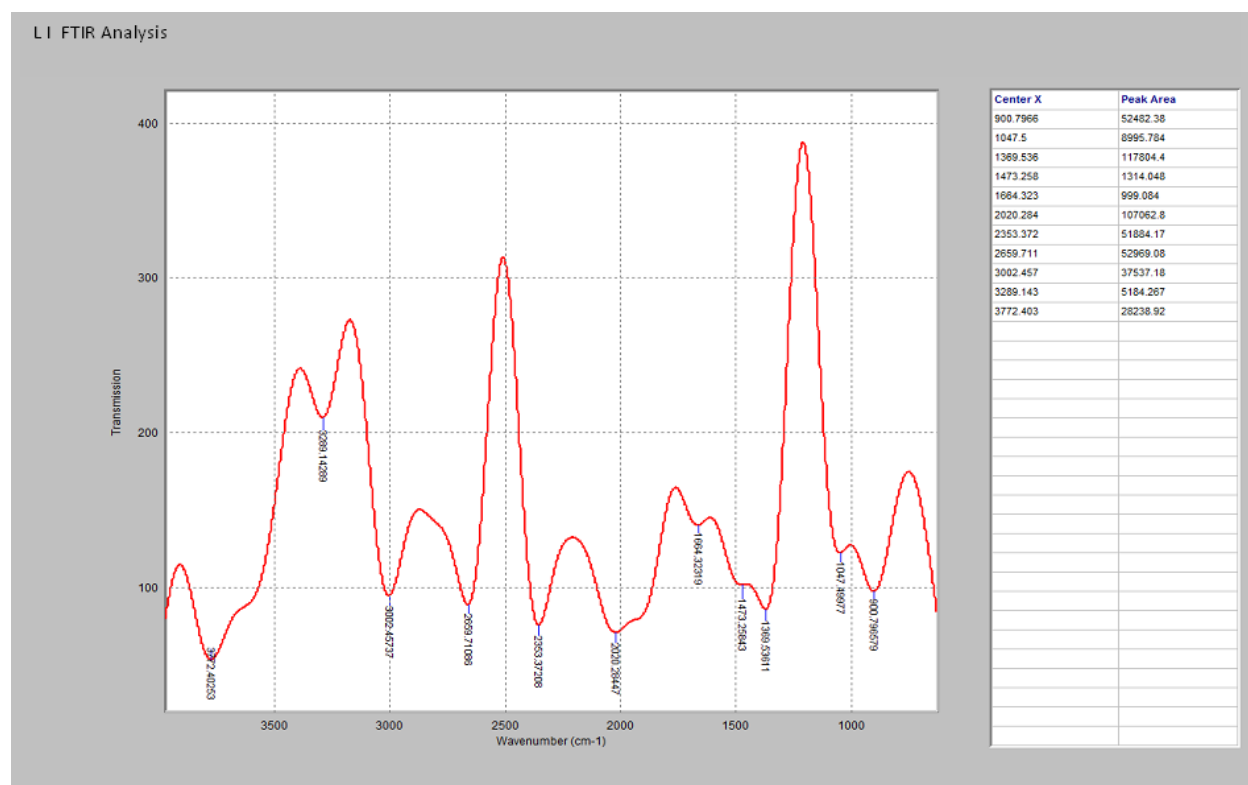
Appendix 2.6 GC-FID Chromatogram For Ethanolic pulp Extract Of *A. Heterophyllus*



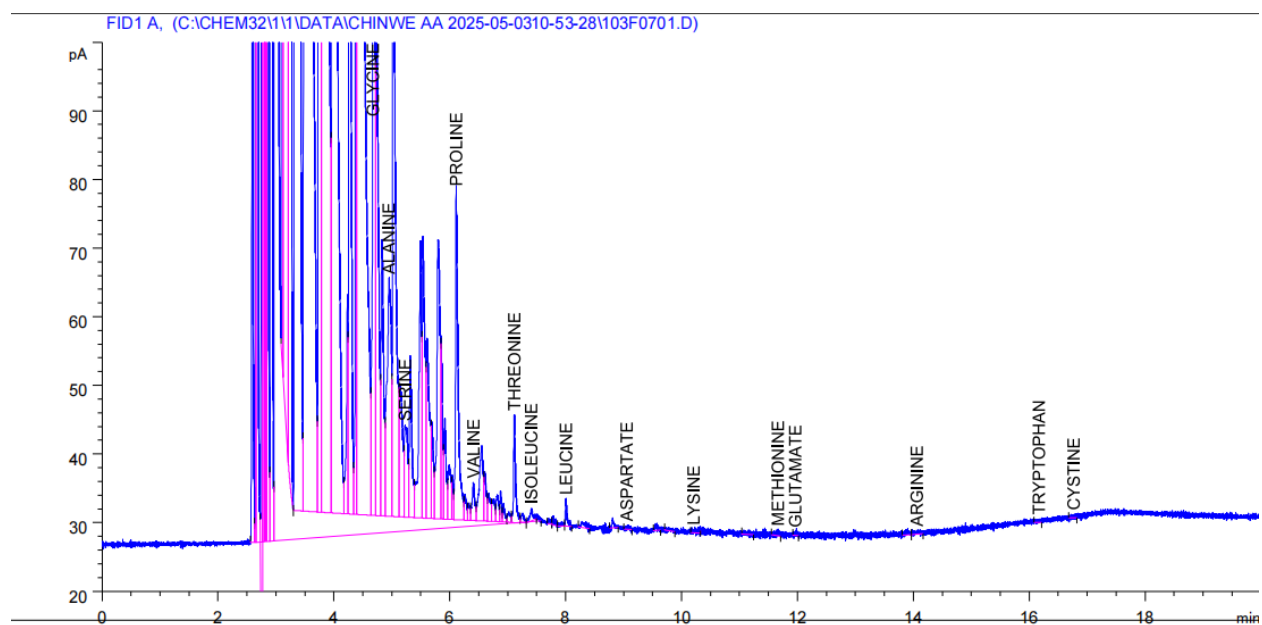
Appendix 2.7 FTIR Chromatogram For Ethanolic Stem Bark Extract Of *A. Heterophyllus*



Appendix 2.8 FTIR chromatogram for ethanolic leaf extract of *a. Heterophyllus*



Appendix 2.9 Amino acid chromatogram for ethanolic stem bark extract of *a. Heterophyllus*



Appendix 2.10 Amino acid chromatogram for ethanolic leaf extract of *A. heterophyllus*

