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AS MEN OF HONOUR WE JOIN HANDS

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Phytochemical Analysis of Gas chromatography mass spectrometry (GC-MS) and Hepato-renal protective effects of *Terminalia catappa* leaf and seed extracts in BPH-induced prostate in rat's model

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ABSTRACT

Background: Benign prostatic hyperplasia (BPH) is a prevalent urological condition. The medicinal plant *Terminalia catappa* has been traditionally utilized for treating diverse ailments. This study explores the phytochemical profile via GC-MS analysis and assesses the hepatorenal protective properties of *Terminalia catappa* leaf and seed extracts in a rat model with BPH induction.

Methods: BPH was induced in rats using testosterone propionate, followed by oral administration of *Terminalia catappa* leaf and seed extracts (250 and 500 mg/kg) for 28 days. Liver and kidney function were evaluated through enzyme and biomarker assessments. Additionally, histopathological analyses of hepatic and renal tissues were performed.

Results: GC-MS profiling revealed various bioactive compounds, including flavonoids, alkaloids, saponins, tannins, steroids, and glycosides. Administration of *Terminalia catappa* extracts led to significant reductions in prostate weight and epithelial thickness while improving liver enzyme and kidney function markers (ALT, ALP, AST, creatinine, and urea) compared to the control group. Histopathological findings indicated enhanced prostate and liver tissue integrity.

Conclusion: This study highlights the phytochemical composition and the hepatorenal protective potential of *T. catappa* leaf and seed extracts in a rat model of BPH. The findings suggest that these extracts may serve as a promising therapeutic approach for managing BPH and associated hepatorenal dysfunction.

1. Introduction

Benign Prostatic Hyperplasia (BPH) is a common urological disorder affecting millions of men worldwide, characterized by the enlargement of the prostate gland, leading to urinary symptoms, sexual dysfunction, and decreased quality of life[1]. The etiology of BPH is multifactorial, involving hormonal imbalance, inflammation, and oxidative stress [2]. Current treatments for BPH, including pharmaceuticals and surgical interventions, often have limitations and side effects [3] Traditional medicine has long utilized plant-based remedies for various ailments, including BPH. *Terminalia Catappa*, a deciduous tree native to East Asia, has been used in folk medicine for its anti-inflammatory, (not applicable), and anticancer properties [4]. *Terminalia catappa*, a tropical tree belonging to the leadwood family, thrives in warm regions across Asia, Africa, and Australia [5]. This species typically reaches a height of around 35 meters, featuring a structured, symmetrical crown with horizontal branches arranged in distinct tiers. The leaves are large, measuring between 15–25 cm in length and 10–14 cm in width, with a dark green, glossy, and leathery texture. The tree is

monoecious, bearing separate male and female flowers, both approximately 0.039 meters in diameter, with a white-to-greenish hue and an inconspicuous appearance due to the absence of petals. The fruits, classified as drupes, undergo color transitions from green to yellow and eventually red upon ripening, enclosing a single seed [6]. Often referred to as the tropical almond, *T. catappa* produces edible fruits commonly consumed by children, birds, and various animals. The kernels are rich in proteins and lipids, making them a valuable food source [7]. Traditionally, the fallen leaves are utilized for brewing herbal teas, widely recognized for their medicinal properties. In regions such as India, the Philippines, and Malaysia, these teas have been employed as remedies for diarrhea and fever, while in Taiwan, they have been associated with liver tumor prevention and hepatitis treatment [8].

Regarding industrial applications, isolating the edible components of *T. catappa* broadens its use as a raw material for food enrichment and supplementation [8]. Advanced processing techniques facilitate fraction separation, allowing transformation into flour, pulp, and oil. These derivatives have potential applications in baked goods, as well as ongoing research into their functional properties. Consequently, interest in this species has expanded to pharmaceutical, chemical, and oleochemical industries [9].

Consuming the seed kernel in moderation has been linked to improving sexual dysfunction, particularly premature ejaculation. Additionally, ethanolic leaf extracts from *T. catappa* exhibit protective effects against osmotically induced hemolysis of human erythrocytes in a dose-dependent manner. The presence of punicalagin and punicalin compounds contributes to the treatment of dermatitis and hepatitis, owing to their potent antioxidant properties [10]. Recent studies have highlighted the potential of natural products in preventing and treating BPH. Phytochemicals from plants have been shown to modulate hormonal balance, reduce inflammation, and exhibit antioxidant activity, thereby alleviating BPH symptoms[11.] GC-MS analysis has emerged as a valuable tool for identifying and quantifying bioactive compounds in plant extracts [12]

This study aims to investigate the phytochemical composition, GC-MS profiling, and hepatorenal protective effects of *T. Catappa*, leaves and seed extracts in BPH-induced prostate in rat models. Our research seeks to contribute to the development of novel, plant-based therapeutics for managing BPH and related hepatorenal complications.

2 Materials and Methods

Aldrich Sigma Chemical Company (St. Louis, MO, USA) provided Ellman's reagent. For the estimation of urea, uric acid, and creatinine, Randox Laboratories Limited (Crumlin, UK) biochemical test kits were used instead of Lab Kit Biochemical Kits (Barcelona). All other chemicals and reagents, with the exception of those noted otherwise, were of the analytical grade and were bought from British Drug Houses in Poole, UK.

2.1 Tropical almond seed kernel and leaves

Fresh ripened tropical almond fruits and fresh leaves were collected from staff quarters of University of Calabar, Calabar and were authenticated at the Department of Botany, University of Calabar where a voucher specimen of the leaves was deposited at the herbarium (voucher No : Herb/Bot/UCC/ 080). The ripen fruits were cracked open and seed kernel removed, washed and weighed. Also, the leaves were washed in clean water and dried alongside the seeds under shade for about 2 weeks. Both the dried seeds and leaves were pulverized and milled into powder using a blender to obtain 0.65kg of seed and 0.8kg of leaf powder preparatory for extraction.

2.3 Preparation of Extracts For the extraction process, 100 g of *Terminalia catappa* seed kernel and leaf powders were separately soaked in 100 ml of 95% ethanol, with intermittent stirring throughout the duration. After 48 hours for the seed kernel and 28 hours for the leaf extract, the mixtures were filtered using Whatman filter paper (No. 1). The filtrates were then concentrated using a rotary evaporator at 30°C six hours for the seed kernel extract and four hours for the leaf extract. The resulting concentrates (14.8 g for the seed extract and 13.6 g for the leaf extract) were stored under refrigeration for subsequent use.

2.4 Acute toxicity testing

An acute toxicity study was done to determine the LD_{50} of both extracts in mice using the Lorke's method [13.] Details of procedure are as presented in appendix.

2.5 Qualitative phytochemical

The qualitative phytochemical screening of the leaves and seed-kernel of *Terminalia. Catappa* was carried out with the methods of [14] and [15.] And the details of the respective principles for the phytochemical are as spelt out below.

2.5.1 Determination of alkaloids: Identification of the presence of alkaloids was according to [16]. Different portions of ethanolic extracts of samples was dissolved in 2 N HCl and treated with few drops of Mayers and wagners reagent respectively. The greenish or brown precipitate indicated the presence of respective Alkaloids.

2.5.2 Determination of tannins: 2grams of Crude dry

powder of each sample was treated with alcoholic $FeCl_3$ reagent. Blue colour indicated the presence of tannins. [17]

2.5.3 Determination of saponins: The presence of saponins was determined by Froths test. Crude dry extract 1g of each sample was vigorously shaken with distilled water and allowed to stand for 15min in a graduated cylinder. A 2cm layer of forth (foam) indicated the presence of Saponin. [18]

2.5.4 Determination of flavonoids: Alkaline reagent test was used in the identification of flavonoids. Aqueous solution of the extract was treated with 10% ammonium hydroxide solution. Yellow fluorescence indicated the presence of flavonoids. [19]

2.5.5 Determination of steroids: Liebermann-Burchard reaction was used in identifying steroids. Chloroform solution of crude dry powder of both samples was treated with acetic anhydride and a few drops of concentrated H_2SO_4 added down the side of the test tube. A blue green ring indicated the presence of terpenoids. [20]

2.6 Determination of cardiac glycosides: Keller-Kiliani test was used for the identification of cardiac glycosides. Appearance of greenish blue color after treatment of sample with 1mL $FeCl_3$ and few drops of conc. H_2SO_4 indicated the presence of cardiac glycosides. [20]

2.6.1 Gas chromatography mass spectrometry analysis

The leaves and seed-kernel extracts of *Terminalia. Catappa* were subjected to GC-MS analysis to identify the volatile bioactive compounds present in the samples. GC/MS analysis on the ethanolic extract was carried out using Agilent 7890A-5975C GC-MS system employing the following conditions.

HP5- column (30 m x 0.25 mm x 0.25 µm), operating in electron impact mode at 70ev Carrier glass flow (1 ml/min), Injection Volume (0.5 µl), split ratio 10:1, injection temperature 250ºc. Interpretation of mass spectrum GC-MS was conducted using the NIST database in the NIST library 2017. The name molecular weight and structure of the components of the test materials were ascertained.

2.6.2 Animal model

Male albino experimental rats ranged in weight from 200 g to 250 g were obtained from our Institution's College of Medicine, housed under ambient conditions in the Departmental animal house, University of Calabar, Calabar, Nigeria. The rats were handled in conformation with the ARRIVE guidelines. [16] We obtained ethical approval (UNICAL/CBS/BCM/SP/2480) from our Departmental Ethical Committee, University of Calabar, Calabar.

2.6.3 Induction of Benign Prostatic Hyperplasia (BPH)

Testosterone Propionate Administration: BPH was induced by subcutaneous injection of testosterone propionate at a dose of 3 mg/kg body weight, administered daily for 4 weeks.

2.6.4 Experimental Design / Animal Grouping From a total of 52 rats induced with BPH, 48 were randomly distributed into eight experimental groups (B–I), each consisting of six rats. Additionally, six non-induced rats were allocated to Group A to serve as the normal control. Group B functioned as the BPH control group, receiving no treatment after induction. Group C was treated with finasteride, a standard anti-BPH drug, at a dosage of 0.1 mg/kg, serving as the standard control. Groups D and E received *T. catappa* leaf extract at doses of 250 mg/kg and 500 mg/kg body weight, respectively. Similarly, Groups F and G were administered *T. catappa* seed extract at 250 mg/kg and 500 mg/kg body weight. Groups H and I received combined extracts of seed kernel and leaves at 250 mg/kg body weight (125 mg from each extract) and 500 mg/kg body weight (250 mg from each extract), respectively.

2.6.5 Biochemical Analysis

2.7 Determination of serum aspartate aminotransfereas (AST) activity

Serum Aspartate aminotransferase (AST) activity was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4 – dinitrophenylhydrazine [21]–

$$\alpha\text{oxoglutarate} + \text{L-aspartate} \xrightarrow{GOT} \text{L-glutamate} + \text{Oxaloacetate.}$$

The concentration of oxaloacetate which is proportional to aspartate consumed by AST and hence its activity was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4 – dinitrophenyihydrazine. Detailed procedures is in appendix.

2.7.1 Determination of serum alanine aminotransferase (ALT) activity

Serum alanine aminotransferase (ALT) activity was measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydraine [22].

$$\text{oxoglutarate} + \text{L-alanine} \xrightarrow{GPT} \text{L-glutamate} + \text{pyruate}$$

Pyruvate whose concentration depends on the amount of L-alanine aminotransferase and hence the activity of ALT was measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine at 546nm. Detailed procedure is presented in appendix.

2.7.2 Determination of serum alkaline phosphatase (ALP) activity

The activity of serum ALP was determined by using the kinetic colorimetric method of optimized Deutaxhw Gesellschaft for Klinische Chemie (DGKC) by the German society of Clinical Chemists, GSCC [23]

Principle: $-p$ nitrophenylphosphate + H_20 $\xrightarrow{ALP}$ phosphate + nitrophenol

Under alkaline condition, colourless nitrophenol is converted to 4 – nitropheoxide, which develops a very intense yellow colour. Its intensity is proportional to the activity of alkaline phosphate in the sample.

2.7.3 Determination of urea concentration

Serum urea concentration was determined using the Agape assay kit method as described by [24]

The principle is given as follows:

$$\text{Urea} + H_20 \xrightarrow{Urease} 2NH_3 + CO_2$$

$$NH_3 + \text{salicylate} \xrightarrow[\text{Hypochlorite}]{Nitroprusside} \text{2, 2-Dicarboxy indophenols}$$

Detailed procedure is as presented in appendix

2.7.4 Determination of serum creatinine concentration

Serum creatinine concentration was determined using the Agape diagnostic kit method as described by [24.]

2.7.5 Histopathological Results

Prostate and liver tissues were fixed in 10% formalin, sectioned, and stained with hematoxylin and eosin (H&E). The histopathological changes were then examined under a light microscope for evaluation.

2.8 Statistical Analysis

The experimental data obtained from this study were subjected to statistical evaluation using analysis of variance (ANOVA) and Student's t-test, performed with SPSS software version 21.0. Results were presented as mean ± standard deviation (SD) for a sample size of six (n=6), with a confidence level of 95% (p-value < 0.05).

3.0 Results

Findings from various analyses conducted in this study, including qualitative phytochemical screening and GC-MS profiling of *Terminalia catappa* leaf and seed extracts, as well as evaluations of different treatments on prostatic function, kidney and liver health, antioxidant levels, lipid profile, and histopathological examination of the prostate, are presented below. The qualitative phytochemical composition of dried leaf and seed powders of *Terminalia catappa* is summarized in Table 1. Alkaloid detection using Mayer's and Wagner's methods revealed a high concentration (+++) in both plant components. Saponins were identified in both extracts, showing a substantial presence (+++) in the seed extract, while the leaf extract contained a moderate level (++). Tannins were highly abundant (+++) in the leaves compared to the seed fraction, where they were present in moderate quantities (++). Similarly, flavonoids and steroids appeared in moderate levels (++) in the leaf extract but were more concentrated (+++) in the seed extract. Glycosides were found in moderate quantities in the seed extract but were not detected (-) in the leaves of *Terminalia catappa*.

TABLE 1

Qualitative phytochemical composition of *Terminalia catappa*

S/N	Phytochemicals	T.C Leaves extract	T.C Seed kernel extracts
1.	Alkaloids	+++	+++
2.	Saponins	++	+++
3.	Tannins	+++	++
4.	Flavonoids	++	+++
5.	Steroids	++	+++
6.	Glycosides	-	++

TC = *Terminalia catappa*, + = Trace Amount, ++ = Moderate amount, +++ = High amount, - = Not detected

GC-MS Chemical Profile of *Terminalia catappa*

The GC-MS analysis of *Terminalia catappa* seed kernel extract revealed a high concentration of unsaturated fatty acids, including palmitic acid esters, steroids, and linoleic acids. Based on peak area percentages, nonanoic acid ethyl ester was the most abundant compound, followed by E-11-hexadecanoic acid ethyl ester, octadecanoic acid ethyl ester, and linoleic acid ethyl ester. Hexadecanoic acid was detected in minimal amounts, alongside other compounds present in very low concentrations. Notably, hexadecanoic and octadecanoic acids exhibit biological activities, including anti-inflammatory, antioxidant, and 5-alpha reductase inhibition[22] additionally, certain unsaturated fatty acids, such as palmitic acid, have demonstrated anti-tumor and antifungal properties.

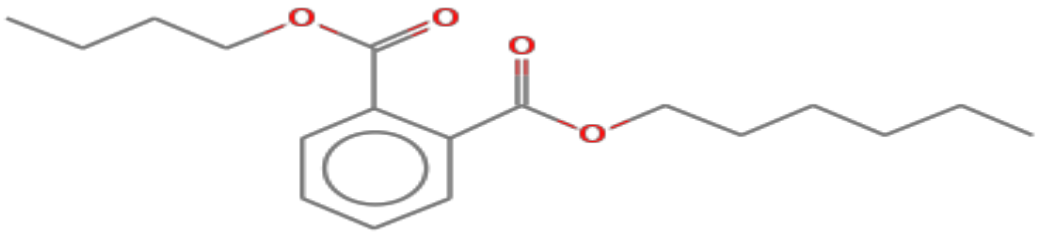
Regarding the ethanolic leaf extract of *Terminalia catappa*, the key bioactive principles, along with their retention times, peak area percentages, molecular formulas, and molecular weights, are detailed in Table 2. The analysis identified eight distinct compounds, with propane-1,1-diethoxy and t-butyl hydrogen phthalate being the predominant constituents. Other compounds, including stigmasteryl tosylate, heptanoic acid ethyl ester, and dodecanoic acid ethyl ester, were found in trace amounts. These compounds contribute to cholesterol metabolism and exhibit anti-cancer properties.

TABLE 2

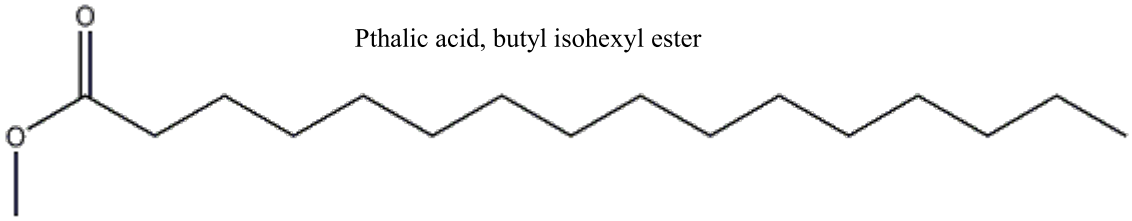
Chemical compounds identified and molecular formula and weight of ethanolic extracts of *Terminalia catappa* seed-kernel

S/N	RT	Name of compound	MF	MW	PA (%)
1	7.270	Phthalic Acid, butyl isohexyl ester	$C_{18}H_{26}O_4$	306	0.52
2.	7.620	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.42
3	7.890	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	4.91
4	8.042	Nonanoic Acid, ethyl ester	$C_{11}H_{22}O_2$	186	61.97
5	8.763	9,12 – Octadecadienal	$C_{18}H_{32}O$	264	0.27
6	8.860	Cis-4-Decenal	$C_{10}H_{18}O$	154	0.395
7	9.278	9,12-Octadecanoic acid, methyl ester	$C_{19}H_{34}O_2$	294	2.223
8	9.295	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308	5.358
9	9.364	E-11-Hexadecanoic acid, ethyl ester	$C_{18}H_{34}O_2$	282	18.360
10	9.541	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	5.578

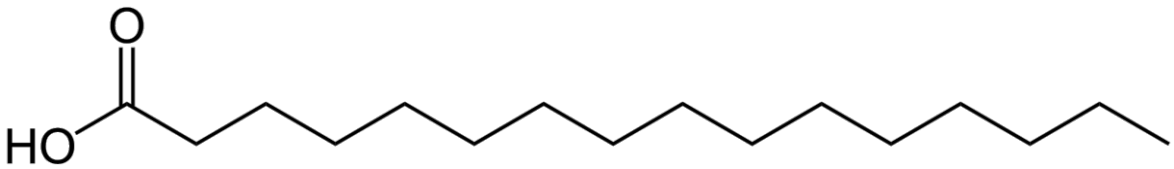
RT: Retention Time, MF: Molecular Formular, MW: Molecular weight, PA: Peak Area percent



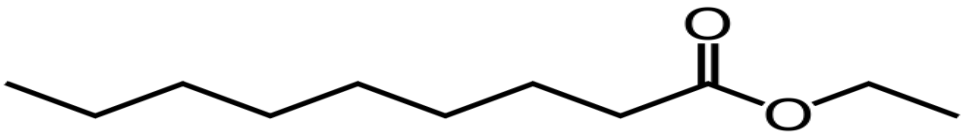
Pthalic acid, butyl isohexyl ester



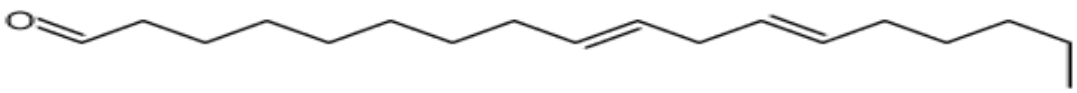
Hexadecanoic acid, methyl ester



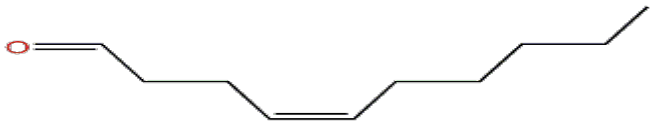
n-Hexadecanoic acid



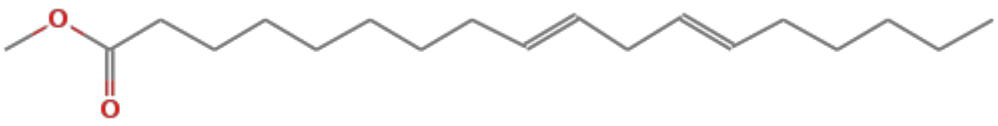
Nonanoic acid, ethyl ester



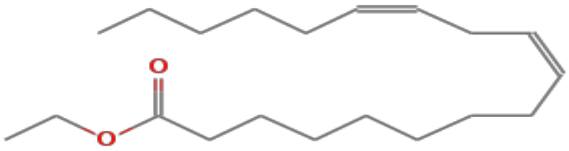
9,12-Octadecadienal



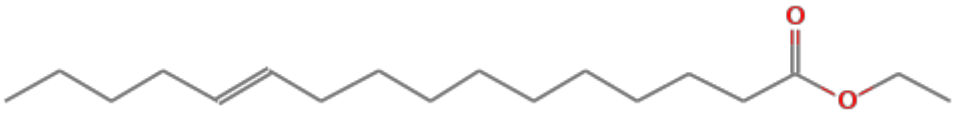
Cis-4-Decenal



9,12-Octadecanoic acid, methyl ester



Linoleic acid ethyl ester



E-11-Hexadecanoic acid ethyl ester

Octadecanoic acid ethyl ester

Effects of Leaf and Seed-Kernel Extracts on Liver and Kidney Function Indices

The impact of *Terminalia catappa* extracts on liver enzymes and kidney function markers in BPH-induced rats is summarized in Table 3. Except for ALP activity, BPH induction led to increased ALT and AST enzyme activity, as well as elevated urea and creatinine levels compared to the normal control group. However, these enzyme elevations did not reach statistical significance ($p<0.05$). Administration of *Terminalia catappa* leaf and seed-kernel extracts, either individually or combined, did not significantly alter liver enzyme activities, including finasteride. Likewise, alkaline phosphatase activity remained unaffected by both BPH induction and *Terminalia catappa* treatment, indicating that liver function was not compromised under BPH conditions and that the extract doses used were within safe limits.

Serum urea concentration showed a statistically significant increase ($p<0.05$) following BPH induction compared to the non-induced control. Treatment with finasteride and *Terminalia catappa* extracts either separately or in combination led to a decrease in urea levels relative to the BPH control. However, this reduction was only statistically significant in the group receiving finasteride, the standard drug ($p<0.05$). Additionally, BPH induction resulted in a mild elevation of serum creatinine ($p>0.05$), which was lowered by finasteride and *Terminalia catappa* extracts, whether administered individually or together. Nonetheless, this reduction in creatinine levels was not statistically significant.

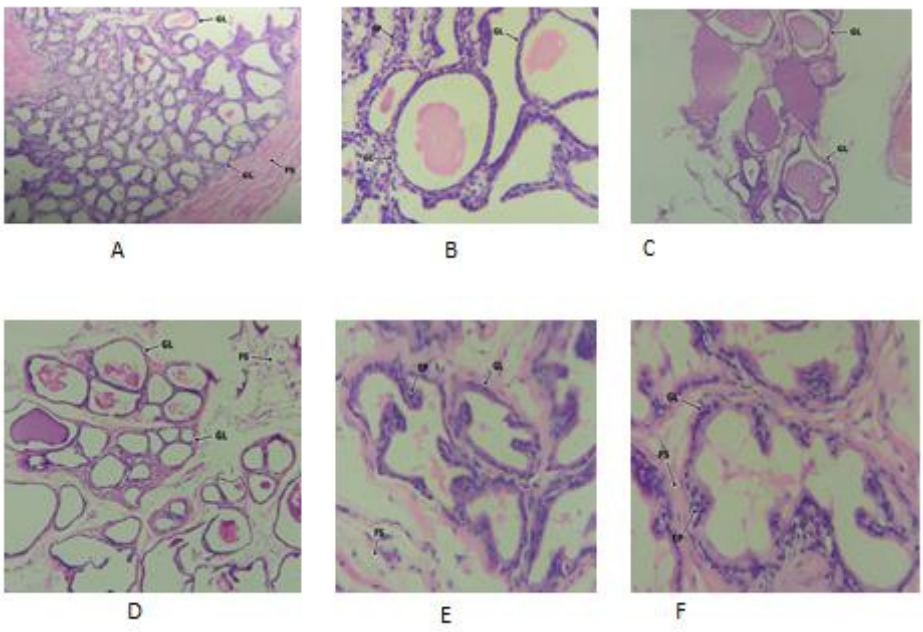
TABLE 3

Effects of *Terminalia catappa* leaf and seed extracts on selected liver and kidney function indices

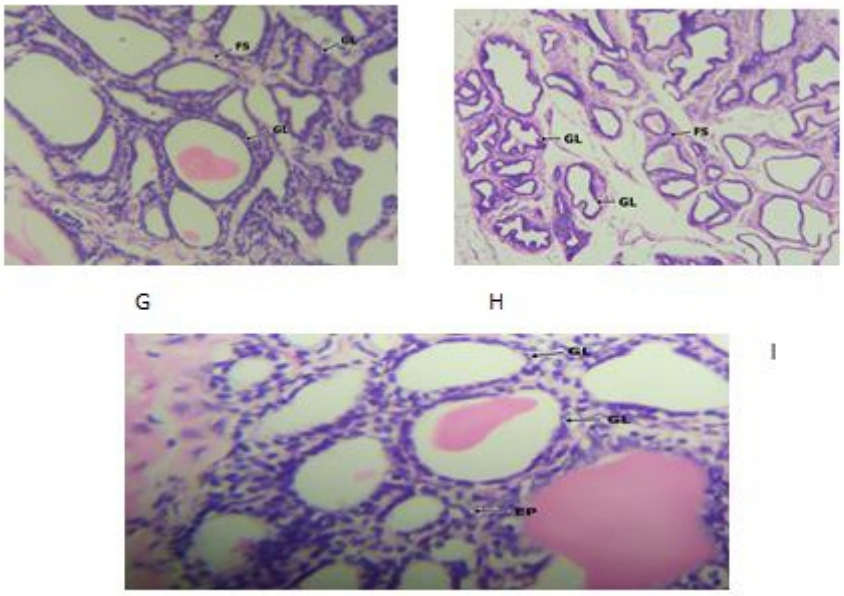
Groups	AST (iμ/l)	ALT (iμ/l)	ALP (iμ/l)	Urea (mg/dl)	Creatinine (mg/dl)
NORMAL CONTROL	54.17±1.22	41.33±2.42	88.67 ± 3.44	27.70±3.40*	1.10±0.04
BPH CONTROL	59.00±1.71	48.50±4.50	76.67 ± 4.89	35.40±3.29	1.55±0.15
BPH + FINESTERIDE	57.17±0.60	45.33±3.77	82.33 ± 8.33	30.83±3.26	1.37±0.15
BPH +250mg leaf extracts TC	58.17±1.62	48.00±5.51	84.50 ± 8.07	33.00±1.70	1.44±0.09
BPH +500mg leaf extracts TC	57.17±1.92	44.83±3.25	90.33 ± 7.23	32.88±2.72	1.51±0.07
BPH +250mg seed extracts TC	57.50±1.64	45.67±4.50	88.00 ± 7.48	33.08±2.92	1.44±0.07
BPH +500mg seed extracts TC	58.67±2.36	45.83±4.12	82.83 ± 6.91	33.23±2.93	1.41±0.14
BPH +125mg (leaf/seed) extracts TC	55.67±1.56	46.83±2.71	83.00 ± 5.97	32.52±2.19	1.42±0.12
BPH +250mg (leaf/seed) extracts TC	57.17±0.95	45.17±4.17	84.17 ± 6.59	33.48±2.26	1.40±0.08

Values are expressed as Mean±SD *P<0.05, n=6

BPH, benign prostatic hyperplasia; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; variable values across and within groups were statistically insignificant.



Magnification x 400, GL-gland, EP-epithelial lining, FS Fibromuscular Stroma
Plate (a) BPH control (b) BPH-induce rat prostate control (c) BPH- induced rat prostate treated with 0.1 mg/kg of finasteride (d) BPH-induced rats treated with 250 mg/kg of *Terminalia catappa* leaf extract (e) BPH-induced rat treated with 500 mg/kg of *Terminalia catappa* leaf extract (f) BPH-induced rat treated with 250 mg/kg



Magnification: 400x GL – Gland **EP** – Epithelial lining **FS** – Fibromuscular stroma

Plate (g) shows prostate tissue from BPH-induced rats administered 500 mg/kg of *Terminalia catappa* ethanol seed extract.

Plate (h) represents prostate tissue from BPH-induced rats treated with a combination of *Terminalia catappa* leaf and seed extracts at a dose of 125 mg/kg.

Plate (i) depicts prostate tissue from BPH-induced rats receiving 250 mg/kg each of both leaf and seed extracts of *Terminalia catappa*.

Histological Changes in Normal and BPH-Induced Prostate

The photomicrograph of the normal control prostate glands (Plate C) displayed uniform cellular structure, with epithelial cells arranged in a tightly packed, double-layered cuboidal formation. A few dilated glands were observed, but most exhibited empty luminal cavities. The intervening stroma appeared thin.

In contrast, the histological examination of the BPH-induced control group (Plate D) revealed irregularly spaced glands varying in size and shape. The stroma was notably dense and fibro muscular, with marked hyperplasia of the epithelial lining. Cystic dilation of the glands was observed, with flattened epithelial cells lining their structure. The luminal cavities contained sparse eosinophilic secretions, confirming the successful induction of BPH and subsequent prostate enlargement.

The histological results in Plates G-I illustrate the effects of Finasteride treatment. Proliferating glands and minimal fibro muscular stroma were noted, along with a noticeable reduction in epithelial cell thickness compared to the normal control (Plate A). The cystically dilated glands exhibited flattened epithelial linings, with some areas showing mild papillary infolding. Eosinophilic secretions were present within the lumen, along with sparse infiltrates. The surrounding fibro muscular stroma was relatively scarce.

Administration of *Terminalia catappa* leaf extracts at 250 mg/kg and 500 mg/kg body weight demonstrated noticeable changes, including proliferating glands separated by limited fibro muscular stroma. The epithelial cells lining the glands appeared flattened, with minimal luminal secretions compared to the BPH-induced control (Plate H), there was a dose-dependent reduction in epithelial and glandular space. However, these effects were less pronounced when compared to the normal control group (Plate I). In the 500 mg/kg *Terminalia catappa* leaf extract-treated group, the luminal cavities contained sparse fluid, though most remained empty. The stroma appeared dense and exhibited scattered inflammatory infiltrates, which were relatively lower in the 250 mg/kg treatment group.

4.0 Discussion

Previous research has demonstrated that plant-based bioactive compounds, particularly flavonoids and steroids, possess anti-inflammatory and anti-proliferative effects, which may contribute to reducing prostate enlargement [25.]

In recent years investigation into organic compounds of plant origin and their activities has been on the rise thus necessitating the development of more specific analytical methods as spectrophotometry, high performance liquid chromatography (HPLC), gas chromatography and chromatography mass spectrometry (GC-MS) [25.] The combination of an ideal separation technique (GC) with the best identification technique (MS) it makes GC-MS a preferred qualitative and quantitative instrument for volatile and semi-volatile compounds whose application cuts across several field of scientific research [26].

In medicine and pharmaceutical practice GC-MS is used in screening for inborn errors of metabolism in newborn and in the detection of oils in Creams, ointments and lotion [27]. GC-MS is exclusively used in bioanalysis of biological fluids (blood and urine) for the presence of barbiturates, narcotics, alcohols, residual solvents, drugs and other food items. The technique can detect adulterations, fatty acid profile, presence of steroids, pollutants and pesticides [28] It Is also extensively used in clinical toxicology for the identification of toxins and venoms and deployed industrially for the analysis of aromatic solvents, inorganic gases, amino alcohols as well as characterization of other industrial compounds [28] GC-MS has now been entrenched in several academic research involving pure and applied sciences, biotechnology and in the advancement of medicinal plant research [33].

This study also indicated a normal liver function with fairly stable liver enzyme activities as shown by the liver enzyme markers AST, ALT and ALP. Though a slightly raised liver enzyme was observed in the BPH control group but differences were statistically insignificant. No substantial evidence has established the relationship between BPH and hepatic dysfunction as BPH may not have any adverse effect on liver function [29]

Treatment with *T. catappa* leaf and seed extracts did not also alter liver function markers AST, ALT, ALP when compared to normal control. *Terminalia catappa* leave extracts has been shown in previous studies to protect against acute liver injury produced by some hepatotoxicants, but the active component and mechanism of actions are not clear [30] though it is suggested that *T. catappa* chloroform extracts provides hepatoprotective effect by the inhibition of the overexpression of interleukin 6 mainly around terminal hepatic vein [31]. This also implies that *T. catappa* had no toxicity effect on the liver. Liver and kidney function markers remained largely stable following the administration of *T. catappa* extracts, reinforcing the plant's safety profile. While BPH induction elevated ALT, AST, and urea levels, extract administration helped

normalize these parameters. This aligns with earlier reports emphasizing the protective effects of medicinal plants on hepatorenal function by modulating oxidative stress and inflammatory responses [10, 31]. The absence of significant liver enzyme alterations suggests that *Terminalia catappa* does not exert hepatotoxic effects, making it a viable candidate for long-term therapeutic application.

Plasma levels of Urea and Creatinine are indicators of kidney functions [32]. This study showed a significant elevation in the urea levels the BPH control group compared with normal controls, though creatinine levels was raised in the BPH control group but difference was not statistically significant when compared to normal control. Although BPH is not a life-threatening condition, the impact of BPH on quality of life (Q.L) can be significant and should not be underestimated [41] The natural history and evolution of benign prostate enlargement ends up in urinary obstruction causing degradation of renal function over time [33].

Gross hematuria with clots with no other identifiable cause is common among BPH patients [34]. This study observed significant association between BPH and renal impairment indicated in the raised levels of urea and creatinine in the raised levels of urea and creatinine in the BPH control group which suggest some degree of renal impairment. This is in line with the findings of [35]. Although the difference in the creatinine levels amongst the groups were not statistically significant as the creatinine levels may become a more significant factor in chronic kidney disease and end organ kidney damage. According to [36] obtaining a serum creatinine measurement may be an appropriate screen for renal diseases unrelated to BPH. The study revealed an improvement in the kidney function with the administration of *Terminalia catappa* extracts in the gradual normalization of the urea levels of the treated groups compared to the BPH control this could be due to the overall improvement of the quality of life previously imparted by BPH. *Terminalia catappa* seed-kernel treatment gave rise to a prostate photomicrograph Section showing widely spaced prostatic glands, cystically dilated and lined by double layered epithelial cells with mucosa infolding's [37] There luminal cavity contains scanty secretion and the separating fibro muscular stroma is scanty. There is also mild inflammatory infiltrates scattered within the stroma with 250mg/kg bw administration.

A more significant reversal in the prostate histology of the 500mg/kg bw *Terminalia catappa* seed-kernel extracts administration plate 1 when compared to the BPH-induced control and even the 250mg/kg bw extracts administration The observed histological improvements further validate the efficacy of these extracts in mitigating pathological changes associated with BPH.. With biphasic proliferation of the glands and fibro muscular stroma. The glands are closely packed and cystically dilated with few containing scanty secretion. The stroma is dense and the mucosa lining are thrown into papillary in folding's. The lining epithelium is columnar cells are relatively smaller compared to the untreated BPH-induced rat prostate, this result is in line with the result of kim et al. [38]

Conclusion

This investigation supports the therapeutic potential of *Terminalia catappa* leaf and seed extracts in alleviating BPH symptoms while maintaining hepatorenal integrity. The presence of bioactive compounds with antioxidant and anti-inflammatory properties further strengthens its medicinal value. However, continued research, including clinical studies, is necessary to optimize its application in human health.

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Conflict of interest

The authors declare no conflict of interest.

DATA AVAILABILITY

Data produced from this study are available from the corresponding author upon reasonable request.

Ethical Approval

The National Academy of Science's (NAS) "Guide for the Care and Use of Laboratory Animals" (National Research Council, 2010) provided guidelines that all of the animals were treated with care. The institution has approved the researcher's experiment under number 17/042144175. University of Calabar, Departmental Ethical Committee, Calabar.

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