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# *In Vivo* and *in Silico* Assessment of Ameliorative Effects of *Xylopia aethiopica* on Testosterone Propionate-Induced Benign Prostatic Hyperplasia

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## Abstract

*Xylopia aethiopica* (XAE) is a commonly used herbal medicine and contains rich active ingredients for a variety of biological activities. The study aimed to explore the role of XAE in the management of benign prostatic hyperplasia (BPH). In the study, testosterone propionate-induced BPH in albino rats was established and treated with different concentrations of ethanol extract of XAE leaf. After treatment, the rats were sacrificed, and the body and prostate weights were recorded. The prostate-specific antigen (PSA) and acid phosphatase (ACP) levels in the blood samples were also determined. Gas chromatography-mass spectrometry was conducted to assess the active chemical compounds. Docking analysis was performed to screen chemical compounds by evaluating their binding affinity with two pro-BPH protein targets (cellular prostatic ACP and PSA). Our data showed the presence of 44 chemical compounds in XAE leaf extract. The body and prostate weights, as well as the levels of PSA and ACP, were significantly increased in BPH induction, and the changing trend was significantly reversed by additional XAE treatment. Interestingly, PSA and ACP levels in XAE-treated groups were reduced to almost the same levels as those in the healthy control. Docking analysis identified four top-posed compounds: β-amyrin, α-amyrin, α-amyrenone, and lupenone with stronger binding energies to prostatic ACP being −9.8, −8.3, −8.4, and −8.6, respectively, compared with the standard drug finasteride (−8.3). Furthermore, the two-dimensional analysis revealed strong interactions through hydrogen bonding, covalent interactions, and several van der Waal forces between the lead compounds and the target proteins. Notably, there was a recurrence interaction between similar residues Asn-1062, Lys-1250, Lys-1059, and Phe-1060 on the protein targets and the lead compounds. The study first revealed the role of XAE in BPH therapy and will help in drug design based on the lead compounds discovered in this work.

**Keywords**
► benign prostatic hyperplasia
► *Xylopia aethiopica*
► acid phosphatase
► prostate-specific antigen
► docking analysis

## Introduction

Benign prostate enlargement is a multifactorial, urological disorder and the commonest noncancerous form of abnormal prostate cell growth affecting older men worldwide.[1] It is an excessive growth that includes nodular hyperplasia, benign prostatic hypertrophy, stromal and glandular epithelial hyperplasia, and benign prostatic hyperplasia (BPH).[2] BPH occurs in the periurethral transition zone of the prostate that surrounds the urethra.[3] BPH is a hormonal, progressive, pathologic condition characterized by the proliferation of prostatic tissues, complex histological changes in the prostate gland, and variable increases in prostate size.[4] It involves prostatitis, fibrosis, the increased adrenergic tone in prostate smooth muscle, and suppression of apoptosis of prostatic cells.[5] The enlarged prostate constricts the urethra, leading to various symptoms, such as a weak urinary stream, incomplete bladder emptying, nocturia, dysuria, and bladder outlet obstruction.[6] The obstructive symptoms when prolonged may eventually lead to acute urinary retention, recurrent urinary tract infection, hematuria, and bladder calculi.[7] When there is no relief, the kidney may get suffocated or drowned in its secretions. Accumulations of urine will result in hydroureter, hydronephrosis, and obstructive nephropathy, and ultimately develops symptoms and signs of renal insufficiency.[7] These symptoms associated with BPH are known as lower urinary tract symptoms (LUTS).[8] LUTS contributes to a pattern of morbidity common in aging men and results in significant annual health care costs in both developed and developing countries.[9]

It is an age-dependent disorder with initial development occurring after 40 years of age and an estimated 50% of men exhibit the symptoms related to BPH by the age of 50.[10] Histological evidence of BPH is found in more than 40% of men in their 50s and nearly 90% of men in their 80s.[11] BPH which causes LUTS increases with advancing age. Moderate to severe symptoms occur in 40 and 80% of men after the age of 60 and by 80 years respectively. Nearly all men develop microscopic BPH by the age of 90 years.[11] The prostate increases in size with passing years but at a decelerating rate. Between the ages of 31 and 50, it doubles in size every 4 to 5 years. Between 50 and 70, this doubling time increases to 10 years, and over 70 till it reaches 100 years.[12] BPH often occurs during the second growth phase. It does not occur in castrated men, but celibates also suffer from BPH.[12]

It has been reported that any of the following treatment options or a combination of two of them might be helpful: surgery, radiation therapy, chemotherapy, α-blockers, hormone therapy, and 5-α-reductase inhibitors, but they all have adverse effects, including but not limited to blood loss, urinary tract infection, and impotence.[13] The commercially available and currently used drugs, 5-α-reductase inhibitors (e.g., finasteride and dutasteride) suppress the dihydrotestosterone (DHT) level by blocking the enzyme 5-α-reductase, resulting in a shrinkage in the size of the prostate, an increase in peak urinary flow rates, as well as a decrease in the level of prostate-specific antigen (PSA), and ultimately providing relief from the symptoms related to the static mechanical obstruction caused by BPH.[14] However, there are salient drawbacks or limitations of finasteride treatment.[15] Therefore, there is a need for complementary alternative herbal medicine that will be helpful to many male sufferers of the debilitating disorder of the prostate gland.[16]

*Xylopia aethiopica* (XAE) is a herb medicine of great repute that produced a variety of phytochemicals including flavonoids and phenols.[17] The rich presence of plant phenolics and flavonoids makes XAE a rich deposit of antioxidants.[18] There has always been a positive association between plant-derived compounds and decreased incidence of chronic diseases due to their antioxidant capacity[19,20] and the presence of abundant phytochemical constituents. In this sense, XAE is a plant with a rich presence of phytochemicals and antiproliferative activity.[17] However, whether and how XAE functioned in the treatment of BPH remained largely unknown. The present study explored the effect of XAE on testosterone propionate (Tp)-induced BPH in albino rats and also identified the potential drugable compounds in XAE that functioned.

## Material and Methods

### Regents

Tp and finasteride were procured from Renhoks Pharmaceuticals, 83 Park Avenue, GRA, Enugu. All other chemicals for the study were of analytical grade.

### XAE Collection and Extraction

XAE leaves were collected from Obukpa Nsukka, Igboeze South Local Government Area of Enugu State, Nigeria. The plant was identified and authenticated by Prof. J. C. Okafor, Consultant Agro Forester and Taxonomist, University of Nigeria, Nsukka.

XAE leaves were washed, dried at ambient temperature for 48 hours, and ground with a manual grinder to a powdered form and sieved. The ground leaves (500 g) were soaked in ethanol (2,000 mL) for 48 hours, allowed to stand, and settled. It was suction-filtered. Repeat the process to ensure maximum extraction. The filtrate was concentrated with a rotary evaporator at 65°C for 3 days. The yield of the extraction was 15%, calculated as a percentage (%), using the formula: weight of extract/weight of sample × 100. The extract product was stored in a refrigerator before use.[21]

### Phytochemical Determination

The total contents of tannin and phenol were determined by a Folin–Ciocalteu method, and the total content of flavonoid was determined by aluminum chloride colorimetric assay, according to Vijay and Rajendra's report.[22] Alkaloid content was determined using the gravimetric method of Harborne.[23] The contents of saponin and cyanogenic glycoside were determined according to Evan's[24] and AOAC's methods,[25] respectively.

### Gas Chromatography-Mass Spectrometry Analysis

The chemical composition of the extract was analyzed using the Agilent GC–MS (Model No. 19091S-933) equipped with

an HP-1MS capillary column (0.25 mm id × 30 m specification length × 0.25 mL film thickness) and an autosampler. The following assay conditions were maintained: 70°C for 2 minutes; raised to 350°C at a rate of 20°C/min and held for 20 minutes; 350°C for MSD transfer line heater; the carrier gas was helium at a flow rate of 1.2 mL/min; split ratio 10:1; run time 32.50 minutes; injection volume 1 µL, Solvent A Washes (PreInj) 2, Solvent A Washes (PostInj) 2, Solvent A volume 8 µL, Solvent B washes (PreInj) 2, Solvent B washes (PostInj) 2, Solvent B volume 8 µL, sample washes 2, sample wash volume 8 µL, sample pumps 6, pressure 4.772 psi, total flow 11.8 mL/min, split flow 8 mL/min scan parameter; low mass 35, and higher mass 550. The constituents were identified by matching the spectra with those found in the NIST II library, and the percentages were computed with the total ion chromatogram.

### Experimental Animals

A total of 200 male Wistar albino rats weighing 250 to 400 g of approximately 16 weeks old were obtained from the Animal House, Department of Biochemistry University of Nigeria, Nsukka. They were maintained under standard laboratory conditions and had free access to feeds and water. The protocol for animal experimentation was performed following guidelines for the care and use of laboratory animals prescribed and approved by the Department of Biochemistry Ethical Committee on Research, Innovation, and Institutional Ethical Committee (EBSU/ET/18/001) of Ebonyi State University Abakaliki.

### Acute Toxicity Test

The lethal dose ($LD_{50}$) of the ethanolic extract of XAE leaf was estimated according to the modified method of Lorke as given by Organization for Economic Co-operation and Development.[26] The study was performed in two phases with 56 male adult albino rats. Phase 1 was made up of five groups: $L_1$, $L_2$, $L_3$, $L_4$, and $L_5$. Each group contained seven rats. Group $L_1$ served as control and they received only the vehicle, olive oil. Groups $L_2$, $L_3$, $L_4$, and $L_5$ were treated with XAE doses of 1,000, 1,500, 2,000, and 2,500 mg/kg body weight intraperitoneally. The second phase of the study comprising three groups $L_6$, $L_7$, and $L_8$ were made up of seven rats each. They were treated with higher doses of XAE: 3,000, 3,500, and 4,000 mg/kg body weight of rats, respectively. They were all kept under the same condition. In each group within 24 hours, symptoms and signs of toxicity were observed and noted. This was repeated for all the groups until 100% death was recorded in a group. The $LD_{50}$ was calculated as the square root of the product of the lowest lethal dose and the highest nonlethal dose from the second stage of dosing. $LD_{50}$ was calculated according to the following Equation. (1):

$$LD_{50} = \sqrt{D_0 \times D_{100}} \tag{1}$$

where $D_0$ is the highest dose that gave mortality; $D_{100}$ is the lowest dose that produced mortality.

### Animal Grouping and Treatment

The rats were acclimatized for 2 weeks and they were divided into 12 groups with 12 rats in each group. They were weighed weekly during the experiment.

- Rats in Group A1 served as control and they received only the vehicle, olive oil.
- Rats in Group A2 were administered TP (14 mg/kg body weight) intraperitoneally daily for 4 weeks to induce BPH.
- Rats in Group B1 were given finasteride (F, 10 mg/Kg) along with Tp (14 mg/Kg) for 8 weeks. F is a potent and specific 5-α-reductase inhibitor.
- Rats in groups B2, B3, B4, and B5 were pretreated with ethanolic extract of XAE doses (100, 200, 300, and 400 mg/kg, respectively) for 4 weeks, then followed by the administration of Tp (14 mg/kg) along with XAE (100, 200, 300, and 400 mg/kg) for 8 successive weeks.
- Rats in Group C1 were pretreated with Tp (14 mg/kg), then given F (10 mg/kg) for 8 weeks.
- Rats in Group C2, C3, C4, and C5 were pretreated with Tp (14 mg/kg) for 4 weeks to induce BPH, then, treated with XAE (500, 600, 700, and 800 mg/kg) for 8 weeks. They were all fed with water and feeds.

After treatment, all rats were anesthetized and sacrificed. Blood samples were taken from each of the rats for targeted enzyme assay. Prostates of the rats were immediately dissected out and weighed. The prostates were homogenized and subjected to histological studies.

### Determination of Serum Prostate-Specific Antigen

PSA was determined according to the method of Nilsson et al.[27] Briefly, serum PSA levels were measured with the PSA Elisa kit according to the manufacturer's instructions. The values were expressed as nanograms of PSA milliliter (-ng/mL) of blood.

### Total Acid Phosphatase Assay

The total acid phosphatase (ACP) was determined according to the method of Allen.[28] The enzyme catalyzes the release of inorganic phosphate from the compound of organophosphates at pH 4.9. The liberated nitrophenol is proportional to the ACP activity. For the determination of prostatic ACP, the determination is performed in the presence of tartrate. The prostatic enzyme is inhibited by tartrate. The difference in activity between total ACP and the nonprostatic form gives the activity of the prostate form. The serum ACP levels were measured with the ACP Elisa Kit according to the manufacturer's instructions.

### Histopathological Examination

The histopathological examination was performed according to the method described by Talib and Khurana.[29] Briefly, the prostate tissues excised from sacrificed animals were fixed in 10% formaldehyde for 24 hours. After fixation, the tissues were dehydrated in alcohol starting with 75 to 95% ethanol. Thereafter, they were cleared in xylene and embedded in paraffin wax using a rotary microtome. Specimens were in

sections of 4 μm and they were mounted on clean slides and stained with hematoxylin and eosin.

### *In Silico* Studies

#### Target Preparation/Molecular Docking
The structures of PSA (PDB-ID: **2ZCL**) and prostatic ACP (PDB-ID: 1CVI) were taken from the database of the Protein Data Bank (PDB) (www.rcsb.org). An open-source molecular editor was used to import the structures (Chimera 1.14)[30] in the protein preparation to remove nonstandard residues, minimize the protein energy, and add hydrogen and charges (gastiger) ready for use in the docking process. The ligands were obtained in the structure data file format (sdf) from the PubChem database (pubchem.ncbi.nlm.nih.gov/compound) These ligands were uploaded into PyRx 0.8 software for virtual screening where it was minimized and converted to auto dock PDBQT format before docking. Virtual high throughput screening of 44 compounds plus standard drug finasteride (PubChem CID: 57363) based on binding energy scores with targeted proteins PSA (PDB-ID: **2ZCL**) and prostatic ACP (PDB-ID: 1CVI) was performed with the PyRx software in the PDBQT format.[31] Of the 44 ligands, 4 ligands β-amyrin, α-amyrin. α-amrinone, and lupenone with docking scores −9.8, −8.3, −8.4, and −8.6, respectively, better than standard drug finasteride (−8.3) against prostatic ACP were selected for redocking with AutoDock MGL tool 1.5.6 based on binding affinities. All four selected ligands maintained superior binding affinities on redocking compared with the standard drug. No ligand was selected as a possible target against PSA due to its overall low binding affinity toward it. A grid box was produced with the values $X = 21$, $Y = 18$, and $Z = 30$ for docking with 2ZCL, while that for prostatic ACP was set at $X = 25$, $Y = 19$, and $Z = 29$.

#### Absorption, Distribution, Metabolism, and Excretion/Pharmacokinetics Predictions Analysis
The absorption, distribution, metabolism, and excretion (ADME) and pharmacokinetics predictions analysis of top-posed compounds were performed using the online web server SwissADME (http://www.swissadme.ch/).[32] This Web site uses the canonical smiles of the examined compounds or potential pharmaceuticals to calculate physicochemical descriptors and forecast ADME parameters, pharmacokinetic qualities, drug-like nature, and medicinal chemistry friendliness of one or more small molecules in the drug development process.

#### Molecular Properties and Bioavailability Score Prediction
The molecular properties and bioavailability score were predicted using the mol-inspiration webserver (https://www.molinspiration.com/cgi-bin/properties). This Web site predicts a variety of conceivable drug targets (each of which requires a unique combination of matching molecular features) which is so great that a common denominator may be found for all of them, allowing molecule drug-likeness to be expressed by a single "magic number." Simple count criteria (such as molecular weight restrictions, logP, or the number of hydrogen bond donors or acceptors) have been useful for weeding out obvious non-drugs.

## Results

### Phytochemical Constituents of XAE Leaf Ethanol Extract
The results of the phytochemical constituents of XAE leaf ethanol extract are shown in ►**Table 1**. The result shows that the glycosides recorded the highest ($14.09 \pm 0.09\%$), while the tannins are the lowest ($1.05 \pm 0.02\%$). The other chemical constituents recorded were alkaloids ($1.22 \pm 0.05\%$), flavonoids ($12.43 \pm 0.60\%$), saponins ($11.77 \pm 0.03\%$), phenols ($3.45 \pm 0.10\%$), while steroids were not in detectable amounts.

### LD$_{50}$ Value of XAE Leaf Ethanol Extract in Albino Rats
The extract of XAE was safe as it presented no mortality in experimental rats up to a dose of 3,500 mg/kg body weight. At a higher dose of 4,000 mg/kg body weight, 57.14% mortality was observed. These two close values were used in calculating the LD$_{50}$ value of XAE of 3,741.66 mg/kg body weight.

### Effect of XAE on Body and Prostate Weights in Tp-Induced BPH in Rats
The results of XAE on body and prostate weights of Tp-induced BPH in rats are shown in ►**Table 2**. The body and prostate weights in were significantly ($p < 0.05$) increased in BPH-induced rats (Group A2) when compared with the control group (Group A1). However, with the additional treatment of finasteride and XAE (Group B1–B5, Group C1–C5), the weight gains were significantly ($p < 0.05$) reduced when compared with the corresponding BPH-induced group.

### Effect of XAE on PSA and ACP Levels in Tp-Induced BPH in Rats
The results of XAE on PSA and ACP are shown in ►**Table 3**. The PSA and ACP levels were elevated significantly ($p < 0.05$) in the BPH-induced group (Group A2) when compared with the

**Table 1** Phytochemical constituents of ethanolic extract of XAE

| Phytochemicals | Mean ± standard deviation (%) |
|---|---|
| Alkaloids | $1.22 \pm 0.05$ |
| Flavonoids | $12.77 \pm 0.12$ |
| Saponins | $12.27 \pm 0.09$ |
| Glycosides | $14.09 \pm 0.09$ |
| Tannins | $1.05 \pm 0.02$ |
| Phenols | $3.45 \pm 0.10$ |
| Steroids | ND |

Abbreviation: ND, not detected XAE, *Xylopia aethiopica*.
Note: Data were presented as the mean ± standard deviation of at least three parallels.

**Table 2** Effect of ethanol extract XAE on body and prostate weights in Tp-induced BPH in rats

| Group | Initial body weight (g) | Final body weight (%) | Weight gain (%) | Prostate weight (g) |
|---|---|---|---|---|
| A1 | 240 | 255 | $6.25 \pm 0.47^c$ | $0.624 \pm 0.15^c$ |
| A2 | 260 | 307 | $18.08 \pm 1.42^a$ | $2.374 \pm 0.35^a$ |
| B1 | 200 | 225 | $12.50 \pm 0.75^b$ | $1.497 \pm 0.32^b$ |
| B2 | 280 | 310 | $10.71 \pm 0.22^c$ | $1.404 \pm 0.28^b$ |
| B3 | 300 | 328 | $9.33 \pm 0.20^c$ | $1.305 \pm 0.25^b$ |
| B4 | 280 | 301 | $7.50 \pm 0.04^c$ | $1.252 \pm 0.31^b$ |
| B5 | 270 | 285 | $5.50 \pm 0.02^c$ | $1.215 \pm 0.35^b$ |
| Group | Initial weight (g) | Final weight (%) | Weight gain (%) | Prostate weight (g) |
| A1 | 240 | 255 | $6.25 \pm 0.47^c$ | $0.624 \pm 0.15^c$ |
| A2 | 260 | 307 | $18.08 \pm 1.42^a$ | $2.374 \pm 0.35^a$ |
| C1 | 400 | 462 | $15.50 \pm 0.48^b$ | $1.165 \pm 0.19^b$ |
| C2 | 280 | 305 | $8.92 \pm 0.18^c$ | $0.819 \pm 0.13^c$ |
| C3 | 320 | 345 | $7.81 \pm 0.16^c$ | $0.726 \pm 0.12^c$ |
| C4 | 280 | 300 | $7.14 \pm 0.12^c$ | $0.652 \pm 0.13^c$ |
| C5 | 260 | 275 | $5.76 \pm 0.15^c$ | $0.512 \pm 0.14^c$ |

Abbreviation: BPH, benign prostate hyperplastic; Tp, testosterone propionate; XAE, *Xylopia aethiopica*.
Note: The significance between the finasteride- or XAE-treated group (Group B1–B5) and BPH group (Group A2) in the preventive studies, as well as the F- or XAE-treated group (Group C1–C5) and BPH group (Group A2) in the curative studies was assessed. Values with different superscripts in the same column are significantly different ($p \le 0.05$).

**Table 3** Effect of XAE on PSA and ACP levels in Tp-induced BPH in rats

| Group | PSA levels (ng/mL) | ACP levels (µg/L) |
|---|---|---|
| A1 | $1.27 \pm 0.15^c$ | $2.02 \pm 0.03^b$ |
| A2 | $2.26 \pm 0.25^a$ | $2.73 \pm 0.14^a$ |
| B1 | $1.85 \pm 0.06^b$ | $2.10 \pm 0.10^b$ |
| B2 | $1.67 \pm 0.01^c$ | $2.05 \pm 0.06^b$ |
| B3 | $1.40 \pm 0.07^c$ | $1.98 \pm 0.15^b$ |
| B4 | $1.30 \pm 0.04^c$ | $1.90 \pm 0.07^b$ |
| B5 | $1.28 \pm 0.05^c$ | $1.87 \pm 0.05^b$ |
| Group | PSA levels (ng/mL) | ACP levels (µg/L) |
| A1 | $1.27 \pm 0.15^c$ | $2.02 \pm 0.03^b$ |
| A2 | $2.26 \pm 0.25^a$ | $2.73 \pm 0.14^a$ |
| C1 | $1.80 \pm 0.07^b$ | $2.20 \pm 0.11^b$ |
| C2 | $1.50 \pm 0.09^c$ | $1.98 \pm 10.12^b$ |
| C3 | $1.35 \pm 0.06^c$ | $1.95 \pm 0.54^b$ |
| C4 | $1.25 \pm 0.01^c$ | $1.90 \pm 0.02^b$ |
| C5 | $1.22 \pm 0.05^c$ | $1.85 \pm 0.01^b$ |

Abbreviation: ACP, acid phosphatase; BPH, benign prostate hyperplastic; PSA, prostate specific antigen; XAE, *Xylopia aethiopica*.
Note: The significance between the finasteride- or XAE-treated group (Group B1–B5) and BPH group (Group A2) in the preventive studies, as well as the finasteride- or XAE-treated group (Group C1–C5) and BPH group (Group A2) in the curative studies was assessed. Values with different superscripts in the same column are significantly different ($p \le 0.05$).

control group (Group A1). When the BPH-induced group (Group A2) was treated with finasteride and XAE (B1–B5 groups for the preventive studies, and C1–C5 groups for the curative studies), the PSA and ACP levels were dose-dependently reduced with the maximum reverse to levels comparable to the control.

### Histopathological Studies of Tp-Induced BPH in Rats

The results of the histopathological studies of Tp-induced BPH in rats are shown in Plates 1 (►**Fig. 1**A–F). The histomicrograph of prostate tissue sections in TP-induced BPH rats showed distorted prostate glands of varying sizes, closely packed glands, no intervening stroma, and cystically dilated glands. Whereas the histomicrogram of the prostate section of rats in the control group showed normal histological features, normal and few glands, uniformly sized glands, intact connective tissues, and normal fibromuscular stroma. However, the histomicrogram of prostate tissues of finasteride and XAE treatment groups showed improvement from the disrupted histoarchitecture of Tp-induced BPH rats. There were few prostate glands, normal glands to stroma ratios, and uniformly sized glands.

### Virtual Screening of Bioactive Compounds

Prostatic ACP (PDB ID: 1CVI) exhibited higher binding affinities compared with a PSA (PDB ID: 1ZCL) by displaying overall lower binding energies across all the screened ligands (►**Table 4**). However, only the top four posed compounds with binding energies lower than the standard drug finasteride used in the management of BPH were selected for
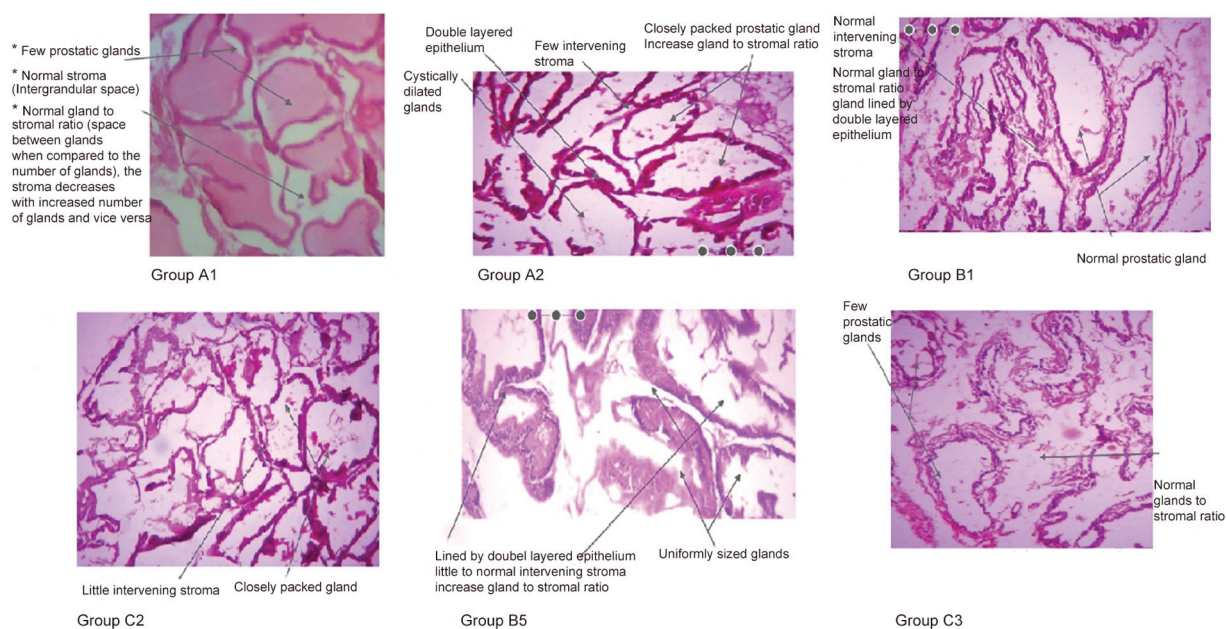
**Fig. 1** Histological studies of the prostate section of rats in different groups.

further *in silico* docking analysis. The lead compounds were β-amyrin (**43**), α-amyrin (**44**), α-amyrenone (**35**), and Lupenone (**27**) and showed the following binding energies (binding affinities): −9.8, −8.3, −8.4, and −8.6, respectively, compared with the standard drug finasteride (−8.3) against prostatic ACP.

The structures of the lead bioactive compounds are presented in ►**Fig. 2**. The stereo configurations in the structures of α- and β-amyrins indicated a difference in the stereochemical arrangements of the methyl groups at positions 29 and 30 of the fifth ring. One notable and unique structural feature present in the selected compounds and the standard drug is the possession of a pharmacophoric center at the C3 position of their carbon skeleton, basically, carbonyl and hydroxyl groups. These groups formed the observed stable hydrogen bond between the target prostatic ACP.

### Analysis of the Binding Interactions of Lead Bioactive Compounds

The analyses of the two-dimensional (2D) and three-dimensional (3D) diagrams illustrating the bonding interactions of the top-posed bioactive compounds are shown in ►**Figs. 3** to **7**. Molecular docking analysis revealed strong interactions through hydrogen bonding, covalent interactions, and several van der Waal forces between β-amyrin, α-amyrin, α-amyrenone, and lupenone similar to the observed stable bonds between finasteride (standard drug), and prostatic ACP (target protein). Notably, across the entire top-posed bioactive compounds, there was a recurrence interaction between similar residues Asn-1062, Lys-1250, Lys-1059, and Phe-1060 on the protein target prostatic ACP.

### Analysis of the Drug-Likeness, Absorption, Metabolism Profile, and Molecular Properties

Drug-likeness of the lead compounds was analyzed using Lipinski's rule of five, Ghose, and Verber's rule, and the results were shown in ►**Table 5**, while the absorption and metabolism profiles of these compounds are presented in ►**Table 6** with the molecular properties given in ►**Table 7**. Our data showed that there were different degrees of violation of the three rules for drug-like properties. Similarly, there was a gradation in their human gastrointestinal tract absorption profile from low to high. These ligands also exhibited varying degrees of cytochrome-P variant inhibition profiles with also different molecular properties including topological polar surface area (TPSA) and miLogP. The result indicated that these top-posed compounds had low HGIT absorption, not P-GS, and were not transmittable via the blood–brain barrier (►**Table 6**). More so, the compounds were not inhibitors of the Cyt-P450 superfamily of enzymes (►**Table 6**). The compounds all had a relative molecular mass of <500 and TPSA within acceptable ranges far less than 90 (►**Table 7**), indicating they are good drug candidates. The bioavailability scores of these ligands also indicated that they were all nonion channel ligands but were G protein-coupled receptors and nuclear receptor ligands, and are all nonkinase inhibitors (►**Table 8**).

### Discussion

XAE showed the presence of carbohydrates, ash, fiber, moisture, protein, and fat. The high fiber content may support the activities of XAE against BPH development. This finding is in line with an earlier report that dietary fiber through the consumption of fruits and vegetables is preventive and

**Table 4** Gas chromatography-mass spectrometry analysis of XAE; PubChem IDs of library-identified compounds and docked scores against target proteins

| SN | $R_t$ | Area (%) | Library ID | PubChem CID | PDB ID: 2ZCL | PDB ID: 1 CVI |
|---|---|---|---|---|---|---|
| 1 | 2.968 | 0.38 | 1-Pentanol | 6276 | −0.1 | −5.6 |
| 2 | 5.720 | 0.06 | 3-Hexenoic acid | 5282708 | 12.1 | −5.3 |
| 3 | 7.946 | 0.03 | Verbenyl, ethyl ether | 567757 | 149.4 | −4.5 |
| 4 | 8.307 | 0.06 | *D*-Verbenone | 29025 | 31.4 | −5.7 |
| 5 | 8.358 | 0.08 | (−)-Myrtenol | 88301 | −1.1 | −5.2 |
| 6 | 9.291 | 0.11 | 2'-Hydroxy-5'-methylacetophenone | 15068 | −3.9 | −5.7 |
| 7 | 10.086 | 0.06 | Tricyclo[3.3.1.1(3,7)]decane-2,6-dione, 4-(methylamino)- | 551123 | 121.7 | −7.2 |
| 8 | 10.344 | 0.07 | Caryophyllene | 5281515 | 178 | −6.1 |
| 9 | 10.452 | 0.05 | 1,6,6-Trimethyl-7-[(*Z*)-3-oxobut-1-enyl]-3,8-dioxatricyclo[5.1.0.0(2,4)]octan-5-one | 5371272 | 17.2 | −4 |
| 10 | 10.801 | 0.09 | 2,6,10,10-Tetramethylbicyclo[7.2.0]undeca-2,6-diene | 5367602 | 8.5 | −5 |
| 11 | 10.933 | 0.06 | Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4aα,7α,8aβ.)]- | 931 | −4.7 | −5.3 |
| 12 | 10.996 | 0.06 | Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2α,4aα,8aβ)]- | 931 | −4.7 | −5.3 |
| 13 | 11.156 | 0.07 | 3-Cyclopentyl-1-propanol | 69842 | −3.9 | −5.3 |
| 14 | 11.316 | 0.07 | Dodecanoic acid | 3893 | 78.1 | −6.3 |
| 15 | 11.465 | 0.77 | 2,5-Dimethoxy-4-ethylamphetamine | 27402 | 18 | −6.3 |
| 16 | 11.694 | 0.21 | Diethyl phthalate | 6781 | 24.8 | −5.1 |
| 17 | 11.757 | 0.07 | Cyclooctene | 638079 | −2.5 | −5.8 |
| 18 | 12.112 | 0.46 | Alloaromadendrene | 91354 | 19.9 | −6.7 |
| 19 | 12.300 | 0.57 | 1,4-Dimethyl-8-isopropylidenetricy clo[5.3.0.0(4,10)]decane | 609236 | 30.3 | −6.6 |
| 20 | 12.403 | 0.49 | Aromandendrene | 91354 | 19.6 | −6.6 |
| 21 | 12.638 | 1.40 | 1,4-Dimethyl-8-isopropylidenetricy clo[5.3.0.0(4,10)]decane | 609236 | 31 | −5.8 |
| 22 | 12.844 | 1.70 | 1*H*-Benzimidazole | 5798 | −4.4 | −5.6 |
| 23 | 13.050 | 3.25 | Pyrimido[1,2-a]indole | 13143755 | 4.7 | −5.3 |
| 24 | 13.245 | 3.94 | Acridin-9-yl-(4-methoxy-phenyl)-amine | 43640 | 13.3 | −4.5 |
| 25 | 13.428 | 5.87 | 3-Octadecyne | 548889 | 1.7 | −5.6 |
| 26 | 13.594 | 5.55 | 1,4-Dimethyl-8-isopropylidenetricy clo[5.3.0.0(4,10)]decane | 609236 | 20.4 | −5.3 |
| 27 | 13.731 | 7.16 | Lupenone | 92158 | 24.4 | −8.6[a] |
| 28 | 13.823 | 5.37 | 2(1H)-Phenanthrenone | 588210 | 38.7 | −4.8 |
| 29 | 13.874 | 8.20 | Lupenone | 92158 | 24.4 | −4.2 |
| 30 | 14.246 | 0.90 | *n*-Hexadecanoic acid | 985 | −3.5 | −6.4 |
| 31 | 14.452 | 0.34 | Hexadecanoic acid, ethyl ester | 12366 | −3.6 | −6 |
| 32 | 14.532 | 0.14 | Alloaromadendrene oxide-(1) | 528759 | 10.2 | −8.3 |
| 33 | 14.658 | 0.07 | 2-Cyclohexene-1-carboxaldehyde, 2,6-dimethyl-6-(4-methyl-3-pentenyl) | 10931390 | 0.7 | −6.1 |
| 34 | 14.761 | 0.34 | 4,6,6-Trimethyl-2-(3-methylbuta-1,3-dienyl)-3-oxatricyclo[5.1.0.0(2,4)]octane | 5369926 | 24 | −5.1 |

**Table 4** (*Continued*)

| SN | $R_t$ | Area (%) | Library ID | PubChem CID | PDB ID: 2ZCL | PDB ID: 1 CVI |
|----|----|----|----|----|----|----|
| 35 | 14.898 | 0.38 | α-Amyrenone | 124018 | 146.1 | −8.4[a] |
| 36 | 15.453 | 18.83 | Phytol | 5280435 | 5.1 | −7.9 |
| 37 | 15.671 | 2.03 | (*E*)-9-Octadecenoic acid propyl ester | 5463340 | −2.9 | −5.5 |
| 38 | 15.842 | 2.66 | Octacosane | 12408 | 3.5 | −5.8 |
| 39 | 16.804 | 5.82 | γ-Sitosterol monohydrate | 133082557 | 13.4 | −4.9 |
| 40 | 17.055 | 7.66 | β-Sitosterol trimethylsilyl ether | 582434 | 9.2 | −5.5 |
| 41 | 17.519 | 5.18 | Oleamide | 5283387 | 0.8 | −6.2 |
| 42 | 17.759 | 1.52 | β-Sitosterol trimethylsilyl ether | 582434 | 9.2 | −7.1 |
| 43 | 18.761 | 4.40 | β-Amyrin | 225689 | −1.9 | −8.3[a] |
| 44 | 19.567 | 0.05 | α-Amyrin | 225688 | −1.9 | −9.8[a] |
| 45 | | | Finasteride (Standard drug) | 57363 | −8.2 | −8.3 |

Abbreviation: XAE, *Xylopia aethiopica*.
[a]Top-posed compounds.
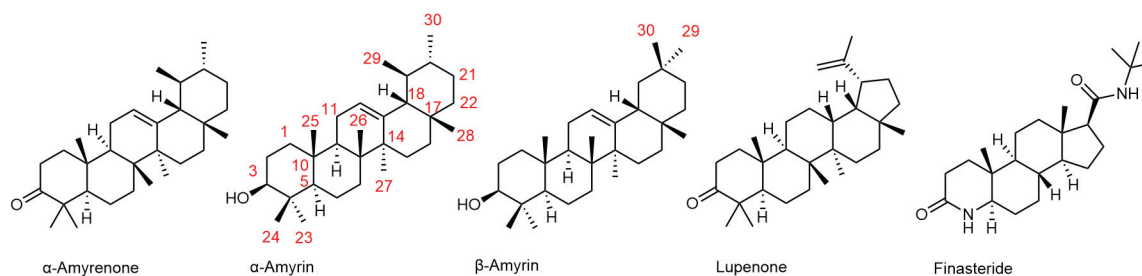


**Fig. 2**  Structures of lead compounds (α-amyrenone, α-amyrin, β-amyrin, and lupenone), and finasteride.
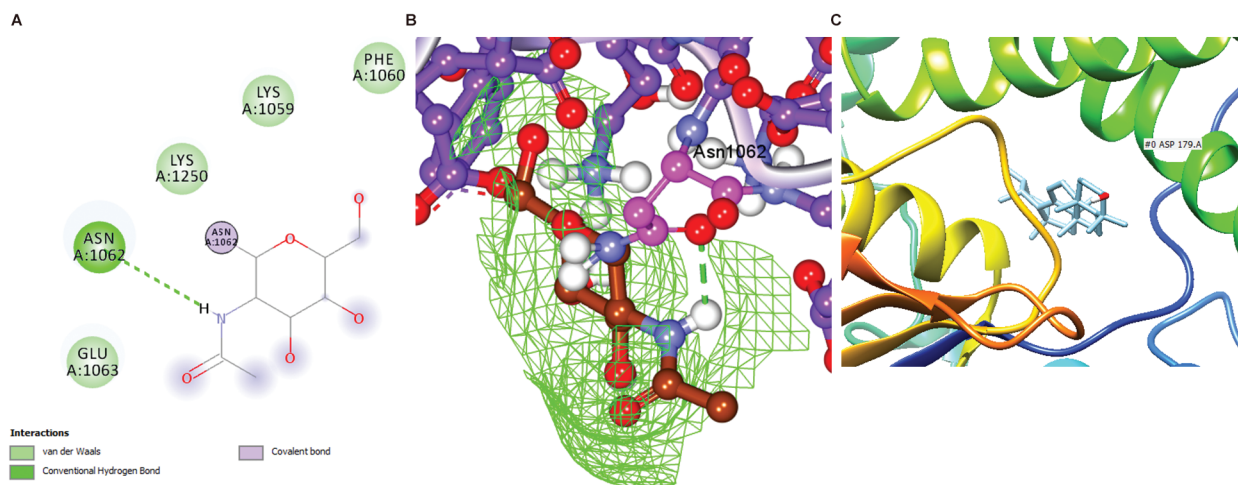


**Fig. 3**  (**A**) 2D and (**B**) 3D structural as well as (**C**) ribbon presentation of β-amyrin-prostatic ACP complex. ACP, acid phosphatase.

associated with decreased incidence of BPH.[33] The phytochemical constituents of XAE also showed the presence of glycosides, flavonoids, saponins, phenols, alkaloids, and tannins. BPH is associated with aging and cellular damage caused by increased oxidative stress.[34] Our result showed a significant presence of antioxidants in XAE, such as flavonoids, saponins, and phenols, which play vital roles in human health[35] and exhibit a wide range of biological effects.[36] The treatment of Tp-induced BPH rats with XAE may have hindered cellular damage induced by oxidative stress due to BPH induction. This is in agreement with Prasad et al' report that several plants have been found to reduce oxidative stress in testosterone-induced BPH in rats.[37] Also, it is consistent with Salah et al' report that flavonoids
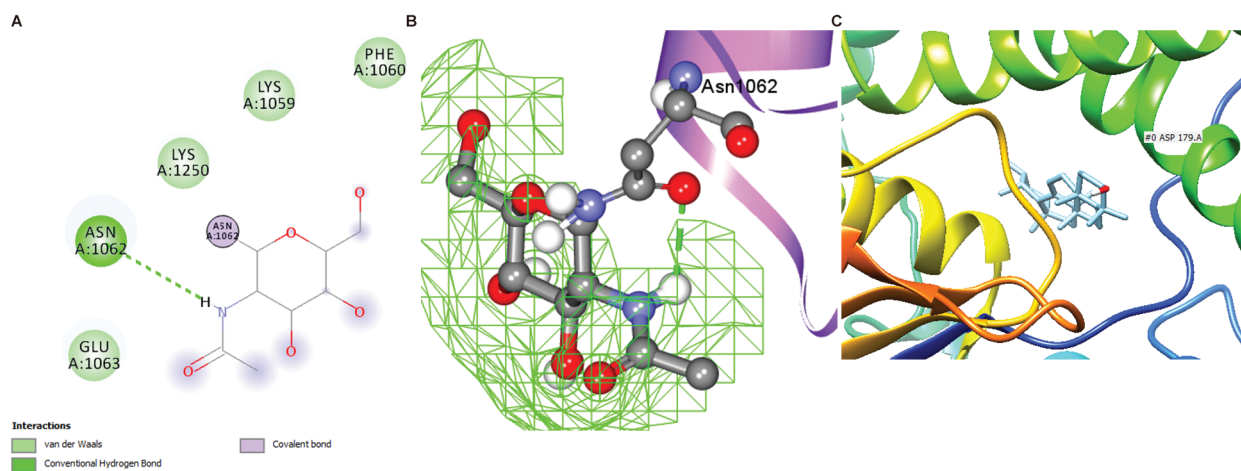
**Fig. 4** (**A**) 2D and (**B**) 3D structural as well as (**C**) ribbon presentation of α-amyrin-prostatic ACP complex. ACP, acid phosphatase.
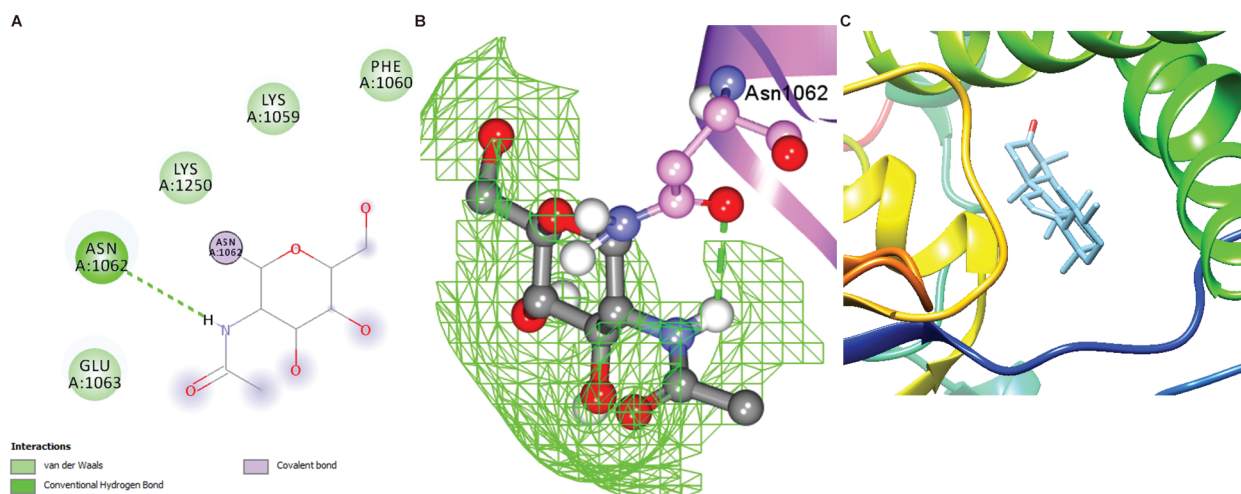


**Fig. 5** (**A**) 2D and (**B**) 3D structural as well as (**C**) ribbon presentation of α-amyrenone-prostatic ACP complex. ACP, acid phosphatase.



**Fig. 6** (**A**) 2D and (**B**) 3D structural as well as (**C**) ribbon presentation of lupenone-prostatic ACP complex. ACP, acid phosphatase.
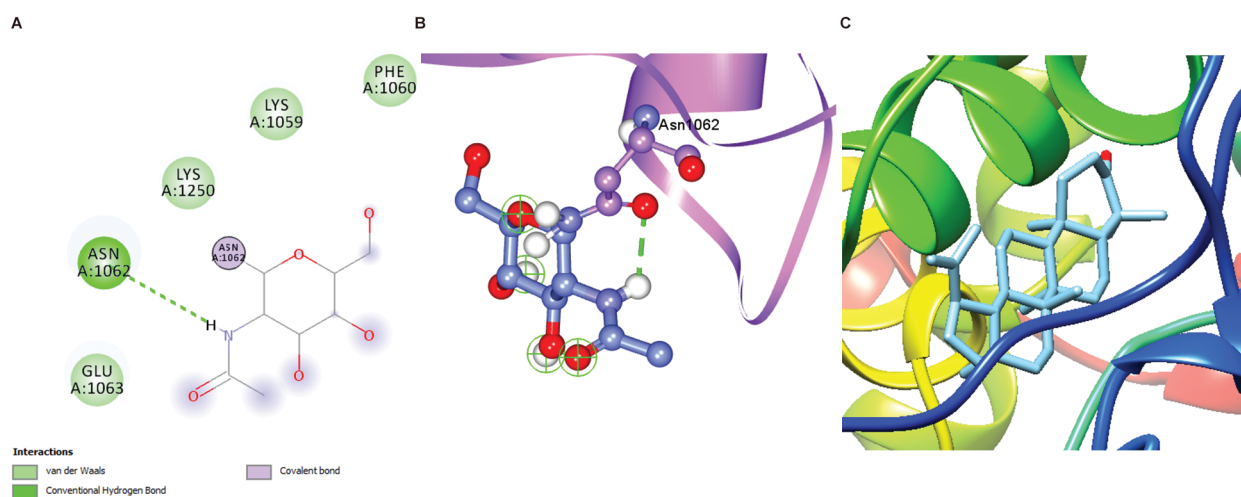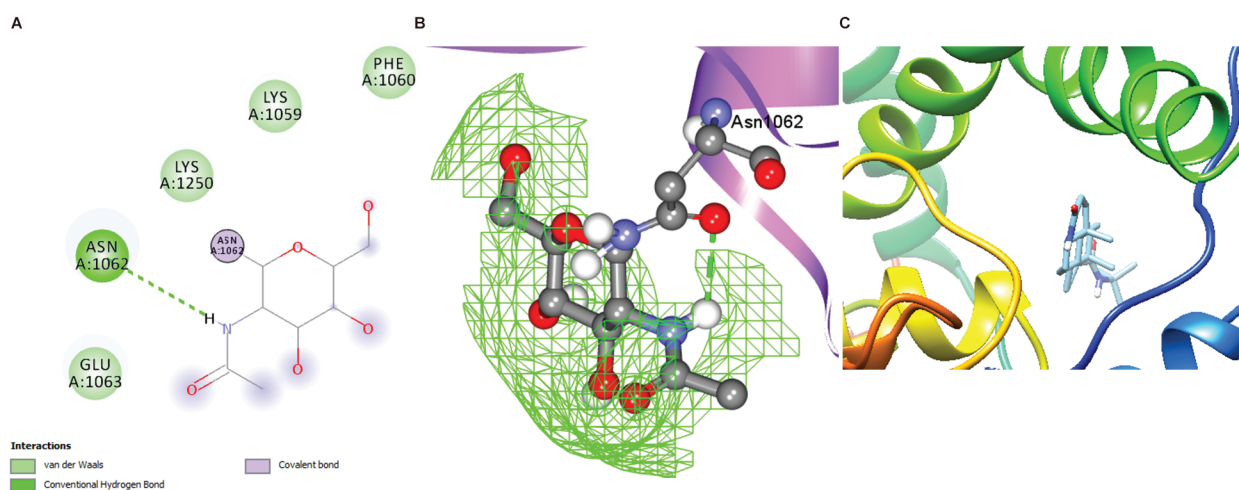
**Fig. 7** (A) 2D and (B) 3D structural as well as (C) ribbon presentation of finasteride-prostatic ACP complex. ACP, acid phosphatase.

**Table 5** Drug-likeness profile of top-posed compounds

| Compound | Mol. wt. | iLogP | HBA | HBD | Lipinski violation | Ghose violation | Verber violation |
|----------|----------|-------|-----|-----|-------------------|-----------------|------------------|
| β-Amyrin | 426.72 | 4.77 | 1 | 1 | 1 | 3 | 0 |
| α-Amyrin | 426.72 | 4.74 | 1 | 1 | 1 | 3 | 0 |
| α-Amyrenone | 424.7 | 4.58 | 1 | 0 | 1 | 3 | 0 |
| Lupenone | 424.7 | 4.54 | 1 | 0 | 1 | 3 | 0 |
| Finasteride | 372.54 | 3.32 | 2 | 2 | 0 | 0 | 0 |

Abbreviations: HBD, H-bond donors; HBA, H-bond acceptors; Mol. wt, molecular weight.

**Table 6** Absorption and metabolism profiles of top-posed compounds

| Compound | HGIA | BBB | P-G S | CYP1A2 inhibitor | CYP2C19 inhibitor | CYP2C9 inhibitor | CYP2D6 inhibitor | CYP3A4 inhibitor |
|----------|------|-----|-------|------------------|-------------------|------------------|------------------|------------------|
| β-Amyrin | Low | No | No | No | No | No | No | No |
| α-Amyrin | Low | No | No | No | No | No | No | No |
| α-Amyrenone | Low | No | No | No | No | No | No | No |
| Lupenone | Low | No | No | No | No | No | No | No |
| Finasteride | High | Yes | Yes | No | No | No | No | No |

Abbreviations: BBB, blood–brain barrier; HGIA, human gastrointestinal absorption; P-GS, P-glycoprotein substrate.

**Table 7** Molinspiration molecular properties of top-posed compounds

| Compound | miLogp | TPSA | natoms | nON | nOHNH | nviolations | nrotb | Volume |
|----------|--------|------|--------|-----|-------|-------------|-------|--------|
| β-Amyrin | 8.02 | 20.23 | 31 | 1 | 1 | 1 | 0 | 460.79 |
| α-Amyrin | 7.90 | 17.07 | 31 | 1 | 0 | 1 | 0 | 455.19 |
| α-Amyrenone | 8.02 | 20.23 | 31 | 1 | 1 | 1 | 0 | 460.78 |
| Lupenone | 8.14 | 17.07 | 31 | 0 | 1 | 1 | 1 | 455.74 |
| Finasteride | 4.00 | 58.20 | 27 | 4 | 2 | 0 | 2 | 376.72 |

Abbreviations: ; miLogP, octanol/water partition coefficient obtained by fitting calculated logP with experimental logP (octanol/water partition coefficient)nrotb, number of rotatable bonds; TPSA, topological polar surface area.
Note: nON and nOHNH derived from Lipinski's rule of 5.

**Table 8** Molinspiration bioavailability score of top-posed compounds

| Compound | GPCR ligand | Ion channel modulator | Kinase inhibitor | Nuclear receptor ligand | Protease inhibitor | Enzyme inhibitor |
|---|---|---|---|---|---|---|
| β-Amyrin | 0.22 | −0.05 | −0.31 | 0.67 | 0.11 | 0.56 |
| α-Amyrin | 0.22 | −0.02 | −0.41 | 0.79 | 0.10 | 0.60 |
| α-Amyrenone | 0.09 | −0.14 | −0.61 | 0.69 | 0.05 | 0.49 |
| Lupenone | 0.14 | −0.01 | −0.62 | 0.75 | 0.01 | 0.41 |
| Finasteride | 0.20 | 0.06 | −0.53 | 0.60 | 0.29 | 0.58 |

Abbreviation: GPCRs, G protein-coupled receptor ligands.

represent one of the most common and widely distributed plant phenolics found in XAE.[38] Flavonoids prevent oxidative cell damage and have strong anticancer activity and cell-protective potentials against all stages of carcinogenesis.[39]

BPH is characterized by stromal and epithelial cell hyperplasia which results in prostate enlargement when sufficiently large, and the prostate constricts the urethral canal to cause partial or sometimes complete obstruction.[40] There have been many studies in the recent past where the measurement of prostate weights has been used to evaluate the inhibitory effects of various substances on the development of BPH.[41] It has already been reported that BPH rats had a significant increase in prostate weights compared with the control group but those treated with finasteride or other herbal remedies had reduced prostate weights compared with the Tp-induced BPH group.[33] These findings in prostate weight results are in agreement with the histopathological studies of the prostate of Tp-induced BPH rats which showed epithelial hyperplasia with an increase in epithelial thickness compared with the control rats. XAE-treated rats showed a reduction in epithelial hyperplasia with a decrease in epithelial thickness.[42] In this study, the observed reduction in prostate weight was similar to the ones shown by finasteride, a 5-α-reductase inhibitor that is suggestive of the inhibitory activity of XAE against the development of BPH (►Table 2).

Serum PSA levels are often elevated in prostate disorders such as BPH and are used as a clinical marker for disease prognosis.[43] Elevated prostate PSA levels promote excessive prostate epithelial and stromal cell growth causing hyperplasia.[44] A decrease in PSA is associated with reduced prostate hyperplasia and 5-α-reductase inhibition.[45] The PSA level of rats in the curative study (Group $C_5$) was reduced to a level ($1.22 \pm 0.05$) significantly ($p < 0.05$) lower than the control value ($1.27 \pm 0.15$) (►Table 3), indicating the potential role of XAE as an effective inhibitor of 5-α-reductase which converts testosterone to a more potent DHT. The result is also in agreement with Akinyemi et al's reports that experimentally induced BPH caused an increase in PSA but reverted to normal value when challenged with methanolic extracts of *Trichosanthes cucumerina* seed and hence prevented BPH development.[46]

ACP is a nonspecific phosphomonoesterase synthesized in prostate epithelial cells and its levels proportionally increase with prostate cancer and BPH progression. It is a key regulator of prostate cell growth[47] and in particular a biochemical marker for the evaluation of excessive prostatic cell growth. ACP is a biomarker that helps rule out cancer of the prostate.[12] However, BPH was the disorder induced in this study. XAE in this study significantly ($p < 0.05$) reduced ACP levels (►Table 3) and may have hindered prostatic cell growth and decreased the incidence of BPH development. The decline in ACP activities further confirms that XAE has chemoprotective effects against BPH proliferation.

XAE and finasteride also improved the histopathological pattern of Tp-induced BPH in rats and showed similar histological features as the control: few prostate glands, normal gland-to-stroma ratio, and uniformly sized glands (►Fig. 1). These findings are in agreement with previous reports of Yeh et al[48] who observed that histomorphology of the prostate of rats with BPH showed epithelial hyperplasia with an increase in epithelial thickness compared with the control; in contrast, with additional treatment of *Yukmijihwang-tang,* mild prostate epithelial hyperplasia with a reduction in epithelial thickness was observed. Our results are also in tandem with that of Emeka and Ogidigo[49] who reported that the Tp-induced BPH group showed an enlarged gland with hyperplastic cells characterized by papillary epithelial cells with vacuolated cytoplasm projecting into the glandular lumen; however, the finasteride group showed reduced hyperplasia while rats fed with *Solanum macrocarpon* leaves showed almost normal prostate.

*In silico* techniques are utilized in the drug development process to indicate a potential drug/ligand mechanism of action and to estimate the tentative binding characteristics of ligand–receptor complexes in advance utilizing a computational approach. In the *in silico* studies, prostatic ACP (PDB ID: 1CVI) exhibited higher binding affinities compared with a PSA (PDB ID: 1ZCL). The current study presents β-amyrin, α-amyrin, α-amyrenone, and lupenone, from XAE, as drug candidates in the management of BPH through the demonstration of stronger binding potentials toward prostatic ACP, a major indicator of prostate health in docking and the absorption, distribution, metabolism, excretion, and toxicity (ADMET) studies, and this could be responsible for the observed modulation of BPH in the animal model of the disease condition. Given that these compounds demonstrated a stronger affinity for the examined protein *in silico* than established conventional modulators of this protein finasteride, this has proved to be viable therapeutic candidates in

the quest for therapies against BPH development and associated medical consequences of finasteride. One of the possible mechanisms of action of these potential drug agents against prostatic ACP is shown in ►**Figs. 3** to **7** and could be based on the premise that these compounds increase prostatic ACP activities by interacting within the androgen-acceptor bonded amino acids of the protein that are similar to those of PSA.[50] This interaction is exemplified by the structure of lead compounds, modeled into the crystal structure of the protein's complexes as indicated by their 3D interactions. These compounds have also exhibited promising drugable properties *in silico*.

## Conclusion

The findings in this study indicated that XAE has preventive and therapeutic effects against Tp-induced BPH in rats like the standard drug finasteride. It is therefore suggestive of inhibitory activities of XAE against 5-α-reductase, a decrease in DHT levels, and shrinkage in the size of the prostate. This study has also presented β-amyrin, α-amyrin, α-amyrenone, and lupenone of XAE as drug candidates in the management of BPH through the demonstration of stronger binding potentials toward prostatic ACP, a major indicator of prostate health in docking and ADMET studies. Hence, the findings indicate that XAE could be very effective in reducing BPH and a panacea for the treatment and management of BPH disorder.

**Conflict of Interest**
The authors have no conflict of interest to declare.

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