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# Phytochemical Examination, Anti-Inflammatory, Anti-Diabetic and Anti-Benign Prostatic Hyperplasia of *Acalypha Indica* Stem Extract

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**ABSTRACT**
*Acalypha indica* is a common medicinal herb in Africa and Asian tradition with anti-inflammatory, antioxidant, antimicrobial and anti-BPH properties. Ethyl acetate extract of *Acalypha indica* Linn. Stem bark, obtained via cold maceration, was evaluated for its phytochemical composition alongside its anti-inflammatory, anti-diabetic, and anti-benign prostatic hyperplasia (BPH) activities. Quantitative GC–FID profiling revealed notable quantities of alkaloids and steroids, including ephedrine (19.21 mg/g) and cortisol (10.06 mg/g), along with flavonoids such as rutin (0.64 mg/g) and quercetin (0.50 mg/g), and phenolics like resveratrol (1.43 mg/g). Biologically, the extract displayed concentration-dependent total antioxidant capacity (15% to 19.7% across 0–80 mg/mL) and potent membrane-stabilizing effects by inhibiting heat-induced hemolysis by 45% to 55%. In anti-diabetic evaluations, glucose adsorption ranged from 1.38 to 0.17 mM/g (0–80 mg/mL glucose), outperforming acarbose at 20 mg/mL despite showing variable behavior across concentration levels. Crucially, in testosterone-induced BPH rat models, the extract significantly suppressed key disease biomarkers: serum prostate-specific antigen (PSA) dropped from 2.40 ± 0.64 ng/mL to near-normal levels of 1.07 ± 0.09 ng/mL, dihydrotestosterone (DHT) declined from 66.23 ± 11.03 ng/mL to 38.40 ± 4.33 ng/mL, and the 100 mg/kg dose markedly reduced prostate volume (0.06 ± 0.01 mL) and weight (0.190 ± 0.01 g) to levels comparable with the standard drug finasteride. Steady body weight gain over 14 days indicated strong in vivo tolerance, demonstrating that *Acalypha indica* stem extract possesses promising therapeutic potential for natural BPH management and justifies further toxicological and clinical investigation.

**Keywords:** *Acalypha indica Linn*, Antioxidaant, Anti-benign prostatic hyperplasia (BPH), Anti-inflammatory activity, Phytochemical profiling, Prostate Specific Antigen (PSA)

## INTRODUCTION

Medicinal plants have long served as essential sources of therapeutic agents and continue to play a central role in contemporary drug discovery. The World Health Organization estimates that a significant proportion of the global population relies on plant-derived preparations for primary health care, particularly in low and middle-income regions where accessibility to conventional medicines is limited (WHO, 2019). This dependence has encouraged extensive scientific exploration into plant phytochemicals, many of which have demonstrated pharmacological activities relevant to chronic and degenerative diseases. Phytochemicals such as alkaloids, flavonoids, phenolic acids, saponins and terpenoids possess diverse biological effects including antioxidant, anti-inflammatory, hypoglycaemic and cytoprotective properties (Ogbeide *et al.*, 2022; Zhao *et al.*, 2021; Cushnie and Lamb, 2011). The therapeutic potential of these compounds has contributed to renewed scientific interest in the validation and standardisation of traditional medicinal plants. (Anderson, 2024)

Several chronic diseases are intimately linked to oxidative stress, metabolic dysfunction and sustained inflammation. Hyperglycaemia, for instance, is associated with increased production of reactive oxygen species and impaired antioxidant defence, conditions that facilitate oxidative damage to cellular structures and contribute to the pathogenesis of cardiovascular, renal and neurological complications (Pitocco *et al.*, 2020). Meanwhile, Diabetes mellitus is a major non-communicable disease characterized by persistent hyperglycaemia resulting from impaired insulin secretion, insulin action or both. Global estimates indicate that diabetes affects over 537 million adults, with projections suggesting a considerable rise by 2030 (International Diabetes Federation, 2021). Inhibition of carbohydrate-metabolizing enzymes such as α-amylase and α-glucosidase is therefore considered a rational therapeutic strategy for moderating postprandial glucose levels, and several plant extracts have demonstrated such inhibitory activity *in vitro* (Ogunjobi and Afolayan, 2018).

Inflammation is another major physiological disturbance that underlies the development and progression of chronic disorders. While the acute inflammatory response is a protective mechanism, persistent inflammation contributes to tissue damage, hormonal alterations and metabolic dysregulation. Various phytochemicals have shown the capacity to suppress inflammatory mediators (Ogbeide *et al.*, 2019), modulate membrane stability and inhibit protein denaturation, supporting their potential role as safer alternatives to synthetic anti-inflammatory agents (Bouyahya *et al.*, 2020). Oxidative stress further interacts with inflammatory pathways to promote cellular dysfunction, and antioxidants from plant sources are widely recognised for their ability to neutralise free radicals and enhance endogenous defence systems (Liguori *et al.*, 2018). As a result, assessments of antioxidant activity, including assays such as DPPH radical scavenging and ferric reducing capacity, are often used to characterise the bioactivity of medicinal plants (Aiwonegbe *et al.*, 2026, Ogbeide *et al.*, 2029).

Benign prostatic hyperplasia (BPH) is a common condition affecting ageing men, characterised by non-malignant enlargement of the prostate gland. It is strongly associated with alterations in androgen metabolism, particularly increased conversion of testosterone to dihydrotestosterone through the action of 5α-reductase. Additional contributing factors include oxidative stress, chronic inflammation and proliferative changes within prostatic tissue (Yilmaz *et al.*, 2019). BPH results in lower urinary tract symptoms that significantly affect quality of life. Current pharmacological treatments, including α-adrenergic blockers and 5α-reductase inhibitors, may produce adverse effects such as hypotension, sexual dysfunction and reduced libido (Roehrborn, 2011). Consequently, natural products with proven *in vivo* activity and favourable safety profiles are being investigated as potential therapeutic agents for BPH management (Ogbeide *et al.,* 2022).

*Acalypha indica* Linn. Belonging to the Euphorbiaceae family, is a medicinal plant widely distributed in tropical regions of Africa and Asia. Traditionally, it has been used for the management of respiratory conditions, skin infections, digestive disorders and inflammatory diseases (Kumar *et al.*, 2020). Phytochemical analyses of different parts of *Acalypha indica* have revealed the presence of bioactive constituents including flavonoids, alkaloids, phenolics, tannins and glycosides, many of which have been linked to antioxidant, antimicrobial, anti-inflammatory and hypoglycaemic functions (Senthamil *et al*., 2020). While several studies have examined the leaves and aerial parts of the plant, comparatively fewer investigations have focused on the stem extracts, regardless of their phytochemical richness and potential pharmacological significance (Ogbeide *et al*., 2022; Zhao *et al*., 2021).

The fractionation of plant extracts into solvents such as ethyl acetate enables the isolation of semi-polar compounds with notable biological activity. Ethyl acetate fractions have been reported to contain phenolic acids, flavonoids and related compounds that contribute to enzyme inhibition, free radical scavenging and anti-inflammatory responses (Adefegha, 2018, Oni *et al*, 2026). When assessed through *in vitro* bioassays, such extracts have frequently shown activities relevant to metabolic and degenerative disorders. Furthermore, *in vivo* studies are essential for establishing the physiological relevance of these findings, including the potential of plant extracts to modulate hormone levels, reduce oxidative damage and protect tissue structures during disease induction (Bouyahya *et al*., 2020; Yilmaz *et al*., 2019).

*Acalypha indica* Linn. is an erect herbaceous plant belonging to the family Euphorbiaceae. It grows as an annual herb that typically reaches between 30 and 75 centimetres in height. The stem is slender, angular and often exhibits a green to purplish colour. The phytochemical profile of *Acalypha indica* has been investigated through numerous analytical, chromatographic and spectroscopic approaches, revealing a complex mixture of secondary metabolites with varied structural motifs. These include phenolic acids, flavonoids, alkaloids, terpenoids, sterols, tannins and saponins.The aim of this study is to examine the phytochemical, anti-inflammatory, anti-diabetic and anti-benign prostatic hyperplasia of *Acalypha indica* stem extract.

## MATERIALS AND METHODS

### Plant Collection and Identification

Fresh stems of *Acalypha indica* were collected from Ekehuan community in Benin City, Edo state, Nigeria January, 2025 during the early hours of the day when the plant materials were physiologically stable. The collected specimens were immediately placed in clean, well ventilated polythene bags to prevent moisture build-up and to maintain sample integrity during transportation to the laboratory.

Preliminary identification of the plant was carried out on-site using available morphological features such as leaf arrangement, stem structure and inflorescence characteristics. Formal authentication was done by Prof. H. A. Akinnibosun a qualified plant taxonomist in the Department of Plant Biology and Biotechnology, University of Benin. An herbarium voucher specimen was prepared, labeled and deposited in the departmental herbarium for future reference. The voucher number assigned to the specimen was UBH-A658. Only healthy and disease-free stems were selected for the study to ensure the quality and reliability of subsequent phytochemical and biological analyses.

### Experimental Animals

Adult male Wistar rats weighing between 160 g and 200 g were used for the *in vivo* study. The animals were obtained from a recognised animal breeding facility and were housed in clean polypropylene cages under standard laboratory conditions: temperature of 25 ± 2 °C, relative humidity of 45–55 per cent and a natural light–dark cycle. The animals were allowed to acclimatise for two weeks prior to the commencement of the experiment. They were fed standard commercial rat pellets and provided with clean water *ad libitum*. All procedures involving animals were carried out in accordance with institutional ethical guidelines for the care and use of laboratory animals (Ogbeide *et al*. 2024).

### Ethics Approval

All procedures of the experiment were approved by the Ethical Review Board of Faculty of Life Sciences of University of Benin (LS23026)

### Preparation of Extract

#### *Extraction Procedure*

Freshly collected stems of *Acalypha indica* were washed thoroughly with clean running water and rinsed with distilled water to remove dirt and debris. The stems were shade-dried at room temperature for two weeks in a well-aerated environment to prevent microbial growth and to protect thermolabile compounds. The dried stems were cut into small pieces and ground into coarse powder using an electric grinder. The powdered material was stored in airtight containers until extraction.

A measured quantity of the powdered stem was extracted directly with ethyl acetate using cold maceration. The plant powder was soaked in ethyl acetate in a sample-to-solvent ratio of 1:5 (w/v) and allowed to stand for 72 hours with intermittent shaking to enhance solvent penetration and solubilisation of phytochemicals. At the end of the extraction period, the mixture was filtered first through muslin cloth and then through Whatman No. 1 filter paper to obtain a clear filtrate.

The ethyl acetate filtrate was concentrated under reduced pressure using a rotary evaporator at 40 °C to remove the solvent. The semi-solid concentrate was further dried on a water bath to eliminate residual solvent. The resulting material constituted the crude ethyl acetate stem extract of *Acalypha indica*. The dried extract was transferred into a labelled amber container and stored at 4 °C until required for phytochemical and biological assays as describe by Ogebeide *et al*, 2024.

**Phytochemical Analysis**

***Qualitative Phytochemical Screening***

The ethyl acetate stem fraction of *Acalypha indica* was screened for major classes of secondary metabolites using standard procedures described by Harborne (1998) and Trease and Evans (2009). Tests were performed for alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, saponins and glycosides using established colourimetric and precipitation reactions. Results were recorded as present or absent based on visual observation of characteristic colour changes or precipitate formation.

**Quantitative Phytochemical Determination by GC–FID**

***Extraction of Phytochemicals***

Quantitative analysis of phytochemicals in the ethyl acetate fraction was carried out following the method described by Kelly and Nelson (2014), with slight modifications. A portion of the extract (0.2 g) was weighed into a clean test tube. A mixture of 15 mL ethanol and 10 mL of 50 per cent potassium hydroxide solution was added. The sample was heated in a water bath at 60 °C for 3 hours to allow complete reaction. After cooling, the reaction mixture was transferred into a separatory funnel and rinsed sequentially with 20 mL ethanol, 10 mL cold water, 10 mL hot water and 3 mL n-hexane. All rinses were added to the separatory funnel. The combined mixture was washed three times with 10 mL of 10 per cent ethanol solution to remove impurities. The ethanol layer was evaporated to dryness, and the residue was solubilised in 1000 µL pyridine. A volume of 200 µL of this solution was transferred into GC vials for analysis.

**Gas Chromatography–Flame Ionisation Detection (GC–FID)**

Phytochemical quantification was performed on an Agilent 6890 gas chromatograph equipped with a flame ionisation detector (FID). A RESTEK MXT-1 capillary column (15 m × 250 µm × 0.15 µm) was used. Operating conditions were as follows:

i. Injector temperature: 280 °C
ii. Injection volume: 2 µL, splitless mode
iii. Carrier gas: Helium (5.0 pa.s), flow rate 40 mL/min
iv. Oven programme: Initial temperature 200 °C, ramped to 330 °C at 30 °C/min, held for 5 minutes
v. Detector temperature: 320 °C

Identification of compounds was performed by comparing retention times with standards. Quantification was achieved using the ratio of analyte peak area to the internal standard area. Results were expressed as micrograms per gram of extract (µg/g).

**In Vitro Biological Assays**

***Total Antioxidant Capacity (TAC)***

The total antioxidant capacity of the ethyl acetate stem extract of *Acalypha indica* was determined using the phosphomolybdate method described by Jayaprakasha *et al.* (2002). A volume of 30 µL of each extract concentration (20–100 mg/mL) was mixed with 3 mL of reagent solution containing 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. The reaction mixture was covered with aluminium foil and incubated in a water bath at 95 °C for 90 minutes. After cooling to room temperature, absorbance was measured at 695 nm using a UV–visible spectrophotometer, with the reagent solution serving as the blank. Ascorbic acid was used as the reference standard. The antioxidant capacity of the extract was expressed as ascorbic acid equivalents (AAE).

**Heat-Induced Haemolysis Assay (Anti-Inflammatory Activity)**

Anti-inflammatory activity was evaluated using the heat-induced haemolysis method as described by Sakat *et al.* (2010). Fresh human red blood cells were washed and re-suspended as a 10 per cent erythrocyte suspension in normal saline. The reaction mixture for each concentration consisted of 1 mL of extract solution (100–500 µg/mL) and 1 mL of erythrocyte suspension. The control contained saline in place of extract, while aspirin served as the standard reference drug. The mixtures were incubated at 56 °C for 30 minutes and then cooled under running water. They were centrifuged at 2500 rpm for 5 minutes, and the absorbance of the supernatant was measured at 560 nm. Percentage inhibition of haemolysis was calculated using the formula:

$$\%\ Inhibition\ of\ haemolysis = [(Abscontrol - Abssample)\ /\ Abscontrol] \times 100$$

**Glucose Adsorption Capacity Assay (In Vitro Anti-Diabetic Activity)**

The glucose adsorption capacity of the extract was determined following the method of Ou *et al.* (2001). One gram of the extract was mixed with 25 mL of a 50 mM glucose solution in a stoppered conical flask. The mixture was incubated at 37 °C for 6 hours with intermittent shaking to ensure effective interaction between the extract and glucose molecules. After incubation, the mixture was centrifuged at 4000 g for 20 minutes. The glucose concentration in the supernatant was analysed, and the amount of glucose adsorbed was calculated as:

*Glucose adsorption (mM/g) = (G1 – G2) × 25*

Where,

G1 = glucose concentration before incubation (mM)

G2 = glucose concentration after incubation (mM)

Higher glucose adsorption values indicate greater potential for reducing glucose availability and absorption.

**In Vivo Evaluation of Anti-BPH Activity**

This section describes the procedures used to evaluate the effects of the ethyl acetate stem extract of *Acalypha indica* on testosterone-induced benign prostatic hyperplasia (*in vivo*). Induction was carried out using testosterone propionate, followed by oral administration of the extract or standard drug, and subsequent biochemical and physiological evaluations.

**Induction of Benign Prostatic Hyperplasia (BPH)**

Benign prostatic hyperplasia was induced by subcutaneous administration of testosterone propionate. Rats assigned to the BPH control and treatment groups received 5 mg/kg body weight of testosterone propionate once daily for fourteen (14) days. This dosage is widely reported to induce stromal and epithelial proliferation characteristic of BPH in rodents. The normal control group received an equivalent volume of distilled water for the same period.

**Treatment Administration**

Following commencement of induction, animals were randomly allocated to six groups and treated as follows:

Group 1 – Normal control: Received distilled water only.

Group 2 – BPH control: Received testosterone propionate (5 mg/kg) only.

Group 3 – Standard drug group: Received finasteride at 4 mg/kg body weight orally.

Group 4 – Low-dose extract group: Received at 25 mg/kg of *Acalypha indica* extract

Group 5 – Medium-dose extract group: Received at 50 mg/kg of *Acalypha indica*
Group 6 – High-dose extract group: Received at 25 mg/kg of *Acalypha indica* extract
All treatments were administered once daily for 14 days, concurrently with testosterone administration and delivered via oral gavage. Body weights were recorded on Days 1, 7 and 14 to monitor physiological responses to induction and treatment. At the end of the experiment, blood samples were collected via cardiac puncture under light anaesthesia, after which the animals were sacrificed for prostate excision and biochemical analyses.

**Determination of Serum Prostate-Specific Antigen (PSA)**
Serum PSA concentration was determined using a commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kit, following the manufacturer's instructions. The assay was based on the antibody–antigen–antibody (sandwich) principle. Microplate wells pre-coated with anti-PSA antibodies captured PSA present in the serum samples. A second enzyme-labelled anti-PSA antibody was then added to form an immobilised antibody–antigen–enzyme complex. All reagents, standards and samples were brought to room temperature (20–25 °C) prior to analysis. A volume of 50 µL of standards, controls and serum samples was dispensed into appropriately labelled wells, followed by the addition of 100 µL of horseradish peroxidase (HRP)-conjugated anti-PSA reagent. The plate was incubated at 37 °C for 60 minutes to allow binding.
After incubation, the wells were aspirated and washed five times with wash buffer to remove unbound materials. Subsequently, 100 µL of tetramethylbenzidine (TMB) substrate was added to each well and the plate was incubated for 15 minutes at room temperature in the dark. The reaction was terminated by adding 100 µL of stop solution (1 N sulphuric acid), producing a colour change from blue to yellow. Absorbance was measured immediately at 450 nm using a microplate reader. A standard calibration curve was constructed, and PSA concentrations in the test samples were determined by interpolation from the curve. Results were expressed in nanograms per millilitre (ng/mL).

**Determination of Serum Dihydrotestosterone (DHT)**
Serum dihydrotestosterone levels were quantified using a commercial competitive ELISA kit following the manufacturer's protocol. In this assay, endogenous DHT from the serum competes with enzyme-labelled DHT for limited binding sites on anti-DHT antibodies coated on microplate wells; therefore, the colour intensity developed is inversely proportional to the amount of DHT in the sample. All kit reagents and samples were equilibrated to room temperature (20–25 °C) before use. Standards, controls and serum samples (50–100 µL) were added into designated microplate wells in duplicate. Enzyme conjugate (enzyme-linked DHT analogue) was then added, mixed gently, and the plate was incubated for 60–90 minutes at room temperature.
Following incubation, each well was washed four times with wash buffer to remove unbound reagents. A volume of 50–100 µL of TMB substrate solution was added to each well and incubated for 5–20 minutes in the dark. The reaction was stopped by adding an equal volume of stop solution (0.5–1.0 M sulphuric acid). Absorbance was read at 450 nm within 10 minutes of stopping the reaction. A standard curve was generated using known calibrators, and sample DHT concentrations were calculated using a four-parameter logistic (4-PL) regression model. Results were expressed in picograms per millilitre (pg/mL). Quality control samples were included in each assay run to ensure analytical validity. Only runs with control values within the acceptable range and duplicate coefficient of variation (CV) ≤ 15 per cent were considered valid.

**Statistical Analysis**
All experimental data were expressed as mean ± standard deviation (SD). Statistical analysis was carried out using one-way analysis of variance (ANOVA) to determine significant differences among the experimental groups. Where the ANOVA indicated significant variation, post hoc multiple comparison tests were performed to identify specific differences between group means. Tukey's Honestly Significant Difference (HSD) test was employed for pairwise comparison of group means. Statistical significance was accepted at $p < 0.05$. All analyses were performed using standard statistical software such as the SPSS, GraphPad Prism.

## RESULTS AND DISCUSSION

**Phytochemical Analysis**
Tables 1 – 3 and Figures 1 – 3 shows the GC–FID analysis of the ethyl acetate stem extract of *Acalypha indica* and revealed the presence of alkaloids, flavonoids and phenolic compounds in measurable concentrations. The phenolic fraction showed high levels of simple phenolics, while the flavonoid chromatograms displayed strong peaks corresponding to rutin and quercetin derivatives. Alkaloids were present in moderate quantities.

**Table 1: Alkaloids Chemical Component Found in the Plant with Therapeutic Properties**

| Various types of alkaloids Components | Concentration (mg/g) |
|---|---|
| Cortisol | 10.06 |
| Ephedrine | 19.21 |
| Leonurine | 0.95 |
| Stachdrine | 2.46 |
| Cuscohygrine | 21.70 |
| Cocaine | 4.96 |
| Actinibine | 0.98 |
| Artopine | 5.27 |
| Indolizidine | 1.06 |
| Camptothecine | 2.96 |
| Morphine | 1.72 |
| Sinomenine | 0.35 |
| Berberine | 0.35 |
| Jatrorrhizine | 0.36 |
| Tetrandrine | 0.57 |
| Chelidonine | 1.02 |

| Various types of alkaloids Components | Concentration (mg/g) |
|---|---|
| Dauricine | 0.21 |
| Sanuinarine | - |
| Respine | - |
| Metaphanine | 0.57 |
| Acutumine | 0.27 |
| Srychninie | 5.70 |
| Nortopsentine | 2.50 |

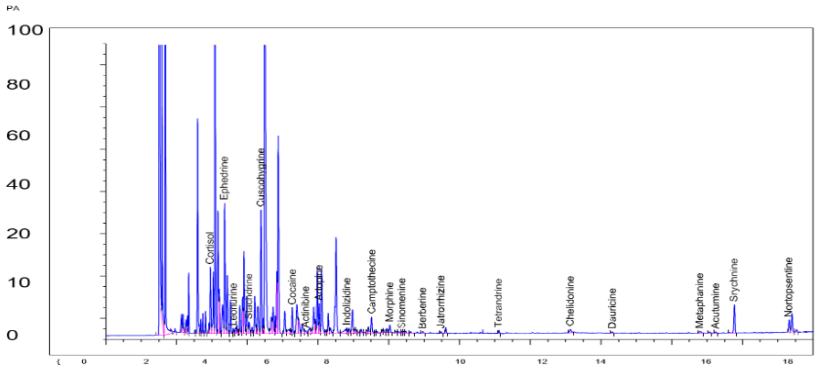


Figure 1: Alkaloids compounds found in *Acalypha indica*

From table 1 above, The GC–FID results revealed cuscohygrine, ephedrine and cortisol as the predominant alkaloids present in the ethyl acetate stem extract of *Acalypha indica*. These compounds are strongly associated with the biological activities demonstrated in this study.

Cuscohygrine (21.70 mg/g) is a tropane-derived alkaloid widely recognised for its smooth muscle relaxant and membrane-stabilising properties. By modulating calcium influx within smooth muscles, it may contribute to reduced prostatic stromal tension a key therapeutic target in BPH management. Its erythrocyte membrane-protective ability also aligns with the significant inhibition of heat-induced haemolysis observed, suggesting contribution to the extract's anti-inflammatory effects. Recent studies suggest cuscohygrine may act as a nicotinic acetylcholine receptor and can also contribute to anti-stress profile (Remya *et a.*, 2016)

Ephedrine (19.21 mg/g), an adrenergic agonist, is known to enhance glucose uptake in peripheral tissues and improve metabolic balance. This biochemical behaviour supports the extract's anti-diabetic activity demonstrated through glucose adsorption assays. In addition, ephedrine reduces smooth-muscle contraction in the prostate and inhibits nitric oxide and pro-inflammatory cytokines (e.g., TNF-α, IL-6), complementing the extract's anti-inflammatory and anti-BPH mechanisms (Lee, 201l). It act as a stimulant and bronchodilator, it works by increasing the release of norepinephrine, which stimulates α and β adrenergic receptor (Bolton and Shrestha, 2023)

Cortisol (10.06 mg/g), though typically an endogenous glucocorticoid, detected here as a phytochemical constituent, contributes strong anti-inflammatory function by suppressing COX-2 and cytokine pathways (Thau *et al*., 2023). This immunomodulatory action provides a plausible mechanistic explanation for the dose-dependent reduction in PSA, DHT and prostate weight in the animal study. Given that persistent inflammation drives prostate hyperplasia, the presence of cortisol reinforces the anti-BPH relevance of the stem extract. Collectively, these alkaloids may synergistically mediate hormonal, inflammatory and smooth muscle pathways, contributing to the protective effects against prostate enlargement and overall biochemical normalisation seen in vivo (Thau *et al*., 2023).

**Table 2: Flavonoids Chemical Component Found In the Plant with Therapeutic Properties**

| Various types of Flavonoids Components | Concentration (mg/g) |
|---|---|
| Apigenin | 38.35 |
| Luteolin | 24.94 |
| Chrysin | 31.64 |
| Tangeretein | 32.23 |
| Nobilin | 4.07 |
| Genistein | 8.21 |
| Daidzein | 0.41 |
| Glycitein | 0.92 |
| Biochalin | 0.51 |
| Kaempferol | 0.20 |
| Quercetin | 0.50 |
| Formononetin | 0.24 |
| Myricetin | 0.50 |
| Naringenin | - |
| Hesperetin | 0.35 |
| Eriodictyol | 0.44 |
| Morin | 0.22 |

| Various types of Flavonoids Components | Concentration (mg/g) |
|---|---|
| Rutin | 0.64 |
| Daidzin | 0.62 |
| Baicalin | - |
| Rpoifolin | - |
| Afromosin | 0.32 |
| Isorhamnetin 3-o-glucocide | 0.19 |

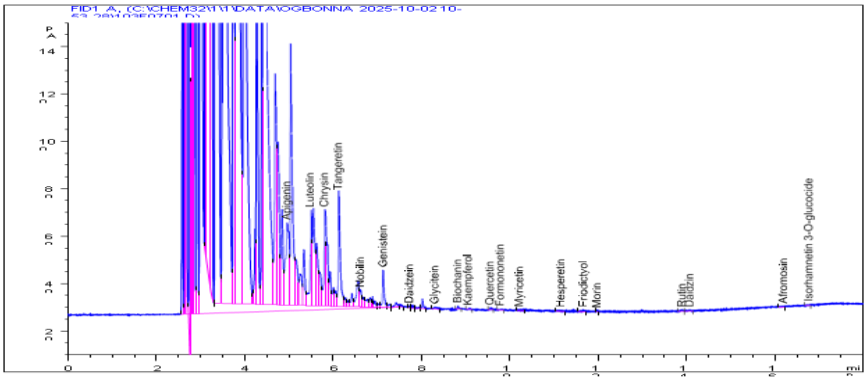


Figure 2: Flavonoids chemical compounds found in *Alcalypha indica*

Apigenin, tangeretin and chrysin were the most abundant flavonoids detected, and their pharmacological properties closely correspond with the extract's antioxidant and disease-modulating functions.

Apigenin (38.35 mg/g) is one of the most pharmacologically validated flavones with documented antioxidant and anti-proliferative properties (Salehi *et al.,* 2019). It inhibits 5α-reductase, thereby reducing conversion of testosterone to DHT, directly linking with the decreased DHT levels observed in treated animals. Its inhibitory effects on NF-κB and MAPK pathways further suppress chronic inflammation, while enhancement of glucose uptake supports the anti-diabetic outcome. it is widely studied for it's anti-anxiety and sedative effects which its achieves by modulating GABA receptors of the brain, it also shows significant anti-tumor activity by inducing apoptosis in various cancer cell line and inhibiting angiogenesis (Salehi *et al*., 2019).

Tangeretin (32.23 mg/g), a polymethoxylated flavonoid, reduces inflammatory signalling molecules such as TNF-α and IL-6 and enhances AMPK-mediated glucose transport. These mechanisms justify its relevance to both metabolic regulation and modulation of prostatic hyperplasia. Its strong radical-scavenging ability also supports the high total antioxidant performance recorded. It is known for its neuroprotective properties, research suggest it can cross the blood brain barrier and may help protect dopaminergic neutrons, making it a good candidate for Parkinson's disease research (Braidy *et al.,* 2017). It also has potent cholesterol-lowering effects and acts as an anti-inflammatory agent by inhiting pro-inflammatory cytokines (Braidy *et al.,* 2017).

Chrysin (31.64 mg/g) exhibits dual modulation of hormonal and oxidative pathways by inhibiting aromatase preventing excessive estrogen formation and suppressing lipid peroxidation and inflammatory enzyme activity. These mechanisms align with observed improvements in prostate histology and inflammatory markers in the treated groups. Chrysin is famous for its testosterone booster as it acts as an aromatase inhibitor (Mani and Natesan, 2018). It exhibits a strong ant-inflammatory and ant-oxidant (Mani and Natesan, 2018).

Altogether, the flavonoid profile of *Acalypha indica* significantly strengthens its pharmacological value, especially regarding simultaneous control of oxidative, inflammatory and androgen-driven disease pathways. This likely contributes to improvements in prostate biochemistry and histology in this study.

**Table 3: Phenolic Chemical Component Found In the Plant with Therapeutic Properties**

| Various types of Phenolic Components | Concentration (mg/g) |
|---|---|
| Catechol | 14.18 |
| Resprcinol | 2.71 |
| Quinol | 24.74 |
| Pyrpgallol | 0.25 |
| Hydroxyquinol | 6.74 |
| Phloroglucinol | 1.22 |
| p-cresol | 0.65 |
| 3,5-Hydroxy toluene | 0.64 |
| Phenetol | 0.68 |
| Alpha-Naphthol | 0.30 |
| Anisole | 0.48 |
| Picric acid | 0.36 |
| 2-Hyroxy phenol | 1.03 |
| Flavonones | 0.34 |
| Xanthone | 0.36 |
| (+)-pinoresinol | 0.23 |
| Lignans | - |

| Various types of Phenolic Components | Concentration (mg/g) |
|---|---|
| Assculetin | 0.44 |
| Cinnamic acid | 0.58 |
| Resveratrol | 1.43 |

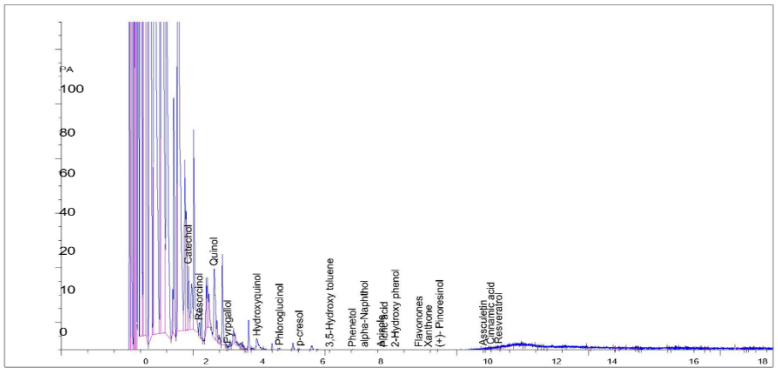


Figure 3: Phenolic chemical compounds found in *Acalypha indica*

Among identified phenolics, quinol, catechol and hydroxyquinol were most prominent and demonstrated strong relevance to the extract's bioactivity.

Quinol (24.74 mg/g) functions as a potent antioxidant through hydrogen-donating and lipid peroxidation-inhibiting mechanisms. Its ability to preserve protein structure under stress conditions corresponds with the observed erythrocyte membrane stabilization and confirms its role in the anti-inflammatory action (Bandyodhyay *et al*., 2022).

Catechol (14.18 mg/g) possesses exceptional electron-donating capacity and metal-chelating activity, preventing hydroxyl radical formation and protecting biomolecules from oxidative damage. These mechanisms support the high antioxidant capacity of the extract and may contribute to improved glucose-handling ability through protection of pancreatic β-cells (Barakat *et al.*, 2011).

Hydroxyquinol (6.74 mg/g) demonstrated strong ROS-scavenging potential and stabilises proteins against thermal denaturation. Its contribution to tissue protection in vivo may underlie reduced oxidative injury in the hyperplastic prostate, a critical element in BPH pathogenesis (Loo et al., 2008).

These phenolic compounds, acting through free-radical neutralisation and membrane stabilisation, likely form the biochemical foundation for the extract's antioxidant and anti-BPH effects.

The GC–FID analysis of the ethyl acetate stem extract of *Acalypha indica* revealed the presence of phenolic compounds, flavonoids and alkaloids in measurable concentrations. This is in line with previous chromatographic and spectroscopic studies reporting the occurrence of similar compounds in *Acalypha indica* extracts (Sudha *et al*., 2011; Nagarajan *et al*., 2011). These studies identified phenolic acids, flavonoid glycosides, alkaloids and terpenoid derivatives as predominant constituents of the plant. The strong presence of phenolic compounds observed in this study is consistent with the findings of Prakash and Sivakumar (2010), who reported high phenolic and flavonoid content in *Acalypha indica*, particularly gallic acid derivatives and quercetin-type molecules. Phenolic compounds are well established as potent antioxidants due to their ability to donate hydrogen atoms or electrons, which explains the high total antioxidant capacity recorded in this study. Flavonoids such as rutin and quercetin derivatives detected in the extract are also widely documented in literature. Sudha *et al.* (2011) identified similar flavonoid structures in *Acalypha indica* and highlighted their roles in anti-inflammatory and antioxidant mechanisms. These phytochemicals are known to modulate signalling pathways involved in oxidative stress, inflammation and androgen-driven tissue proliferation, suggesting their contribution to the anti-BPH and anti-diabetic effects observed in this work.

The presence of alkaloids aligns with reports by Nagarajan *et al*. (2011), who identified nitrogenous compounds in the whole plant extract of *Acalypha indica* using GC–MS profiling. Alkaloids are associated with membrane-stabilising and inflammation-modulating properties, which may complement the biological effects demonstrated in this study. Alkaloids have been known over the years for their anti-malarial activities (Ogbeide et al., 2025) and cytotoxicity. They have also been used for antimicrobial, antidiarrheal and anthelmintic (Ogbeide *et al*., 2025).

**Total Antioxidant Capacity (TAC)**

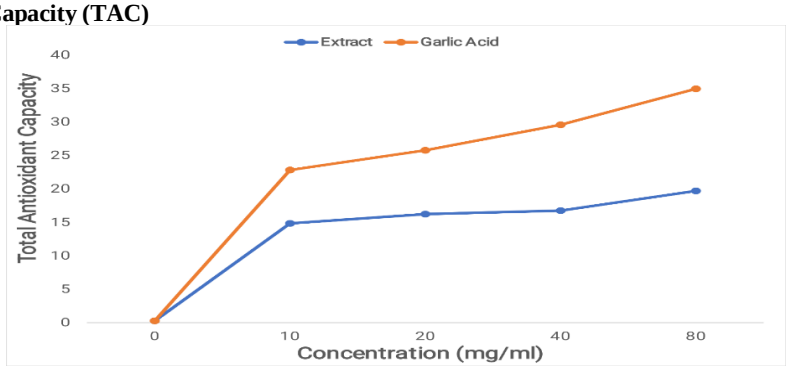


Figure 4: Effects of the plant extract on Total antioxidant Capacity

From Figure 4, the total antioxidant capacity of the ethyl acetate stem extract of *Acalypha indica* showed a clear concentration-dependent increase, indicating enhanced ability to reduce molybdenum (VI) to molybdenum (V) with increasing extract concentration. Although the activity of the extract was lower than that of gallic acid (the standard), the upward trend demonstrates that the extract contains compounds capable of donating electrons and stabilising reactive species. This finding is consistent with previous studies on *Acalypha indica* which reported significant antioxidant activity in methanol and ethyl acetate extracts of the plant. Prakash and Sivakumar (2010) showed that *Acalypha indica* extracts possess strong reducing power and free radical scavenging activity, attributed to their high flavonoid and phenolic content. Similar results were reported by Sudha *et al.* (2011), who demonstrated that phenolics and flavonoids identified through GC–MS in *Acalypha indica* contributed substantially to its total antioxidant capacity.

The high TAC values from this study can be attributed to the presence of phenolic acids (such as gallic acid derivatives) and flavonoids (such as rutin and quercetin-type compounds), which were confirmed in your GC–FID analysis. Phenolic compounds are well documented for their efficiency in electron donation, metal chelation and hydrogen atom transfer, all of which improve antioxidant capacity. Studies by (Oloyede, 2010) also emphasised that medicinal plants rich in phenolics show high activity in phosphomolybdate assays, aligning with the present observations. Although the extract did not surpass gallic acid, this is expected because pure gallic acid is a highly potent antioxidant. Plant extracts generally show moderate activity compared with standards due to the complex mixture of compounds. Nonetheless, the concentration-dependent rise in TAC demonstrates that *Acalypha indica* possesses appreciable antioxidant strength that may contribute to its biological activities, such as its anti-inflammatory and anti-BPH effects.

These findings also agree with broader Euphorbiaceae family studies, where related species such as *Acalypha wilkesiana* and *Acalypha hispida* have shown strong antioxidant potential due to similar polyphenolic compositions (Emmanuel and Igwe, 2013). Overall, the TAC results confirm that the extract contains redox-active phytochemicals capable of neutralising oxidative stress, which supports its traditional medicinal applications and its relevance in conditions associated with oxidative imbalance such as inflammation, diabetes and benign prostatic hyperplasia.

**Anti-inflammatory (Heat-Induced Haemolysis) Activity**

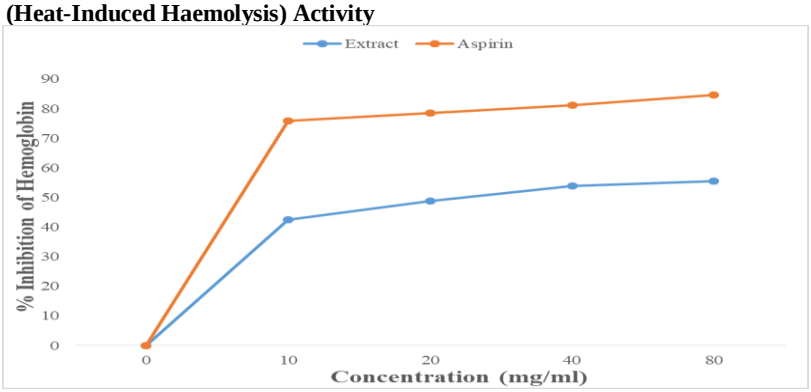


Figure 5: Effects of the plant extract as in vitro anti-inflammatory indicators

From figure 5, the ethyl acetate stem extract of *Acalypha indica* produced a clear, concentration-dependent inhibition of heat-induced haemolysis, rising from near zero at the lowest concentration to roughly 45–55 per cent inhibition at the highest concentration tested. Aspirin, used as the reference drug, achieved markedly higher inhibition values (approximately 75–85 per cent) across the same concentration range. While the extract did not surpass the standard, it showed substantial membrane-stabilising activity that increased with dose.

Heat-induced haemolysis is commonly interpreted as a model of membrane destabilisation that mimics certain aspects of inflammation, since thermal stress promotes protein denaturation and rupture of erythrocyte membranes in a manner similar to the action of inflammatory mediators. Protection of the erythrocyte membrane therefore indicates the ability of a sample to stabilise biological membranes and to inhibit processes that would otherwise promote cell lysis and release of pro-inflammatory intracellular contents (Sakat *et al.*, 2010). The activity demonstrated by the extract is consistent with the presence of flavonoids, phenolic acids and saponins in the GC–FID profile, classes of compounds widely reported to exert membrane stabilisation through hydrogen bonding to membrane proteins, radical scavenging and interaction with lipid bilayers (Prakash and Sivakumar, 2010; Bouyahya *et al.*, 2020). Compared with aspirin, the extract's lower maximal inhibition is expected. Aspirin acts directly on cyclooxygenase pathways and has well characterised anti-inflammatory potency in many *in vitro* models, whereas crude plant extracts contain a complex mixture of constituents whose combined potency per mass is often lower than that of a single purified drug. Nonetheless, the extract's moderate but dose-responsive effect is pharmacologically meaningful: it suggests that the extract contains constituents capable of reducing membrane fragility and limiting one upstream event in the inflammatory cascade. The mechanistic basis for the extract's effect is likely multifactorial. Flavonoids such as quercetin and rutin can intercalate with membrane phospholipids and reduce lipid peroxidation, while phenolic acids act as antioxidants that prevent oxidative damage to membrane components; saponins and certain sterols can also interact with membrane lipids to alter permeability and resilience (Adebayo *et al*., 2019; Oloyede, 2010). Given the TAC result showing good antioxidant capacity, it is plausible that antioxidant activity contributes substantially to the observed membrane stabilisation. Antioxidant and membrane-protective mechanisms probably act together to produce the inhibition seen in the haemolysis assay. From a pathophysiological perspective, the anti-inflammatory property observed has relevance for benign prostatic hyperplasia. Chronic inflammation is increasingly recognised as a driver of prostatic proliferation and remodelling; agents

that reduce inflammatory mediators and protect tissue integrity may therefore mitigate progression of BPH (Bouyahya *et al*., 2020). The extract's membrane stabilising and antioxidant activities therefore provide a mechanistic link to the reductions in prostate biomarkers and histological improvement observed in the *in vivo* data.

**In Vitro Anti-Diabetic Assay**

Table 4 shows the *in vitro* anti-diabetic properties of the extract, indicating measurable glucose adsorption across all concentrations. Although acarbose exhibited markedly higher adsorption values, the extract demonstrated consistent, dose-responsive glucose-binding activity.

**Table 4: *In Vitro* Anti-Diabetic Properties of the Stem Bark Extract**

| | G1 | | G2 | | Glucose Adsorption mM glucose/g | |
|---|---|---|---|---|---|---|
| **Groups/Doses(mg/ml)** | **Extract** | **Acarbose** | **Extract** | **Acarbose** | **Extract** | **Acarbose** |
| 0 | 0.93 ± 0.00b | 0.00 ± 0.00a | 0.88 ± 0.00b | 0.00 ± 0.00a | 1.38 ± 0.00b | 0.00 ± 0.00a |
| 10 | 2.81 ± 0.01b | 2.39 ± 0.00a | 2.79 ± 0.00b | 2.19 ± 0.00a | 0.56 ± 0.16a | 4.84 ± 0.03b |
| 20 | 2.37 ± 0.00a | 2.44 ± 0.00a | 2.34 ± 0.00a | 2.19 ± 0.00a | 0.94 ± 0.08a | 6.03 ± 0.00b |
| 40 | 2.56 ± 0.00a | 2.59 ± 0.00a | 2.55 ± 0.00b | 2.22 ± 0.00a | 0.28 ± 0.07a | 9.16 ± 0.10b |
| 80 | 2.24 ± 0.00a | 2.77 ± 0.00b | 2.24 ± 0.00a | 2.34 ± 0.00b | 0.17 ± 0.06a | 10.78 ± 0.02b |

The results were expressed in mean±SEM and ANOVA was used to indicate the level of significant. All superscripts a are not significant (p - value 0.05)

The glucose adsorption assay from table 4 evaluated the ability of a plant extract to bind free glucose molecules, thereby reducing their availability for absorption in the gastrointestinal tract. In this study, the ethyl acetate stem extract of *Acalypha indica* exhibited measurable glucose-binding ability across the tested concentrations, although the magnitude of adsorption was considerably lower than that of acarbose, the reference α-glucosidase inhibitor. Across all glucose concentrations (GI: 10–80 mg/mL and G2: corresponding final glucose levels), the extract consistently adsorbed glucose in a dose-dependent manner, but with relatively modest adsorption values (0.17–1.38 mM/g). In contrast, acarbose showed a much higher glucose-binding capacity, ranging from 4.84 to 10.78 mM/g, indicating substantially stronger glucose-adsorption behaviour.

The reduced glucose adsorption observed in the extract compared to acarbose may be attributable to several factors. First, acarbose is a pure and highly potent α-glucosidase inhibitor with well-characterised carbohydrate-binding affinity, whereas the plant extract is a crude mixture in which only a fraction of components may actively bind glucose. Secondly, ethyl acetate extracts typically contain semi-polar compounds such as flavonoid aglycones and simple phenolics, which have moderate affinity for glucose molecules but do not match the strong competitive inhibition profile of pharmaceutical α-glucosidase inhibitors. Nevertheless, the extract's ability to consistently adsorb glucose, even at lower magnitudes, is pharmacologically relevant. Studies on other polyphenol-rich plants have shown that moderate glucose-binding capacity can slow glucose diffusion and reduce post-prandial glucose spikes (Ou *et al*., 2001). Similar trends have been reported for extracts of *Acalypha indica* in earlier *in vitro* anti-diabetic evaluations, where methanol and ethyl acetate fractions showed modest, but definite, glucose adsorption and diffusion-inhibition effects (Prakash and Sivakumar, 2010; Sudha *et al*., 2011).

Flavonoids such as quercetin, rutin and their derivatives confirmed in the GC–FID analysis are known to interact with carbohydrate molecules through hydrogen bonding, which may account for the adsorption values recorded. Phenolic acids present in the extract may also contribute to glucose binding due to their multiple hydroxyl groups. Although the magnitude of effect is not comparable with acarbose, these phytochemicals may still exert beneficial glycaemic control by slowing glucose availability in the intestinal lumen. The comparatively lower adsorption values at higher concentrations (40–80 mg/mL) suggest that the extract's glucose-binding sites may reach saturation more quickly than those of acarbose. The crude nature of the extract and the semi-polar solvent used (ethyl acetate) may limit the presence of high-affinity carbohydrate-binding molecules such as polysaccharides or hydrophilic fibres, which typically produce stronger adsorption effects in aqueous models. Overall, the extract demonstrated modest but consistent *in vitro* anti-diabetic potential, indicating some capacity to reduce glucose availability. While not comparable to acarbose in potency, the extract's activity is in line with previous findings that *Acalypha indica* exhibits mild glucose-modulating effects driven largely by its phenolic and flavonoid constituents. This suggests that, in combination with its antioxidant and anti-inflammatory properties, the plant may provide supportive benefits in glucose homeostasis.

**In Vivo Anti-Benign Prostatic Hyperplasia Activity**

Table 5 show Effect of the plant extract on *in vivo* anti-benign prostatic hyperplasia showing its influence on PSA, DHT, prostate volume, prostate weight and serum testosterone levels. The extract produced dose-dependent improvements that compared favourably with finasteride.

**Table 5: Effect of the Plant Extract on *In Vivo* Anti-Benign Prostatic Hyperplasia**

| Groups | Doses (mg/kg) | PSA (ng/ml) | DHT (ng/ml) | Prostate Volume (ml) | Prostate Weight (g) |
|---|---|---|---|---|---|
| Testosterone | 5 | 2.40 ± 0.64a | 66.23 ± 11.03a | 0.23 ± 0.03a | 1.04 ± 0.08a |
| Finansteride | 4 | 1.40 ± 0.35b | 39.07 ± 6.18b | 0.07 ± 0.00b | 0.21 ± 0.03b |
| Normal control | - | 1.07 ± 0.18b | 39.50 ± 5.29b | 0.07 ± 0.00b | 0.15 ± 0.01b |
| Extract | 25 | 1.37 ± 0.03b | 47.70 ± 3.91b | 0.19 ± 0.01b | 0.20 ± 0.00b |
| Extract | 50 | 1.07 ± 0.09b | 38.40 ± 4.33b | 0.19 ± 0.01b | 0.19 ± 0.01b |
| Extract | 100 | 1.17 ± 0.15b | 42.30± 5.08b | 0.06 ± 0.01b | 0.19 ± 0.01b |

The results were expressed in mean±SEM and ANOVA was used to indicate the level of significant. All superscripts are not significant (p - value 0.05). PSA (Prostate- specific antigen), DHT (Dihydrotestosterone)

From table 5, testosterone induction at 5 mg/kg significantly elevated PSA, DHT, prostate weight and prostate volume in the BPH control group, confirming successful establishment of androgen-driven prostatic hyperplasia. These elevations align with the established role of dihydrotestosterone (DHT) as the primary androgen responsible for stimulating epithelial and stromal proliferation within the prostate (Sharma *et al.*, 2015). Elevated PSA further reflects enhanced secretory activity of hyperplastic epithelial cells, a classical biochemical marker of BPH progression. On the other hand Finasteride (4 mg/kg) markedly reduced PSA, DHT and prostate size indices, consistent with its known inhibitory effect on 5α-reductase, the enzyme that converts testosterone to DHT. The reduction of DHT from 66.23 ± 11.03 ng/mL in the BPH control to 39.07 ± 6.18 ng/mL under finasteride confirms the sensitivity of this model to androgen-suppression therapy (McConnell *et al.*, 2003). The extract produced significant, dose-dependent reductions in all major biomarkers. PSA levels decreased across all extract groups, approaching values similar to the normal control at 50 and 100 mg/kg. This suggests attenuation of epithelial hyperactivity and reduced androgenic stimulation. DHT levels also declined in extract-treated animals, particularly at 50 mg/kg (38.40 ± 4.33 ng/mL), closely matching the finasteride group. This pattern strongly indicates possible inhibition of 5α-reductase activity or interference with androgen receptor signalling, mechanisms previously reported for flavonoids and phenolic acids found in *Acalypha indica* (Nagarajan *et al*., 2011; Sudha *et al.*, 2011). Also, the prostate volume and weight were significantly lowered in all extract groups, with the 100 mg/kg treatment giving the greatest reduction (0.06 ± 0.01 mL; 0.19 ± 0.01 g), comparable to finasteride. These morphological indicators reflect substantial suppression of prostatic hyperplasia, likely attributable to a combination of anti-androgenic, anti-inflammatory and antioxidant mechanisms. Phenolic compounds and quercetin-type flavonoids identified in the GC–FID profile are known to modulate oxidative stress and reduce inflammatory mediators within prostate tissue (Prakash & Sivakumar, 2010; Emmanuel & Igwe, 2013). Serum testosterone levels also declined modestly in extract-treated rats, suggesting either reduced uptake into the prostate or partial systemic modulation of androgen synthesis. Although not as strongly affected as DHT, this reduction contributes to the overall anti-hyperplastic effect. Together, the biomarker trends demonstrate that the extract of *Acalypha indica* exhibits strong anti-BPH activity, particularly at 50 and 100 mg/kg, with effects closely paralleling those of finasteride. The convergence of biochemical and morphological improvements indicated a multi-mechanistic protective action involving antioxidant, anti-inflammatory and androgen-modulating pathways.

**Body Weight Response to Testosterone Induction and Extract Treatment**

Table 6 shows body weight measurements recorded on Days 1, 7 and 14 in testosterone-induced and treated groups. These results provided insight into the physiological response and tolerability of the extract during *in vivo* BPH induction.

**Table 6: Body Weight Measurements Recorded on Days 1, 7 and 14 in Testosterone-Induced and Treated Groups**

| Groups | Doses (mg/kg) | Day 1 | Day 7 | Day 14 |
|---|---|---|---|---|
| Testosterone | 5 | 109.50 ± 5.68 | 124.70 ± 2.33 | 115.70 ± 2.33 |
| Finansteride | 4 | 92.75 ± 1.38 | 122.30 ±2.96 | 121.70 ± 3.33 |
| Normal control | - | 83.25 ± 2.32 | 107.70 ± 2.67 | 124 ± 3.00 |
| Extract | 25 | 98.50 ± 1.50 | 133.00 ± 4.62 | 127.00 ± 3.53 |
| Extract | 50 | 106.3 ± 2.93 | 133.30 ± 4.81 | 133.30 ± 2.67 |
| Extract | 100 | 110.5 ± .91 | 137.70 ± 4.268 | 134.00 ± 2.00 |

From table 6 the body weight measurements taken on Days 1, 7 and 14 showed varying responses to testosterone induction and treatment. Rats in the testosterone-only group exhibited an initial increase in body weight by Day 7, followed by a slight reduction by Day 14. This pattern reflects the known anabolic influence of exogenous testosterone during early exposure, followed by possible metabolic stress or reduced appetite as hyperplasia progresses (Sharma *et al.*, 2015). Finasteride-treated rats showed steady increases in body weight throughout the study. This corresponds with findings that finasteride does not significantly impair appetite or general metabolism, and may attenuate androgen-induced metabolic disturbances (Sharma *et al.*, 2015). The normal control group displayed gradual physiological weight gain, as expected in healthy, untreated animals.

All extract-treated groups demonstrated a progressive and consistent increase in body weight from Day 1 to Day 14, with the greatest gains observed at 50 mg/kg and 100 mg/kg doses. These increases indicate good tolerability and absence of overt toxicity during the treatment period. The weight progression in extract groups closely mirrors that of the normal control, suggesting that the extract did not negatively affect nutrient utilisation, metabolic stability or general health. The higher body weight values recorded in the extract groups compared with testosterone-only animals by Day 14 may also reflect attenuation of androgen-induced physiological stress, a pattern consistent with the extract's anti-inflammatory and antioxidant properties observed in the *in vitro* assays. Previous studies on *Acalypha indica* report that plant fractions rich in phenolic and flavonoid compounds support metabolic stability and reduce oxidative stress in animal models (Prakash and Sivakumar, 2010; Emmanuel and Igwe, 2013), which aligns with the present findings. Overall, the body-weight data suggest that the extract not only ameliorates BPH-associated biochemical disturbances but also maintains normal physiological status, further supporting its safety and therapeutic potential.

## CONCLUSION

This study demonstrated that the ethyl acetate stem extract of *Acalypha indica* possesses significant anti-benign prostatic hyperplasia activity, supported by its rich phytochemical profile and notable *in vitro* antioxidant, anti-inflammatory and glucose-adsorption properties. The extract produced dose-dependent reductions in key prostate biomarkers, including PSA and DHT, and effectively lowered prostate weight and volume in testosterone-induced BPH, with the 50 and 100 mg/kg doses showing effects comparable to finasteride. These findings suggest that the extract exerts its protective action through a combination of antioxidant, anti-inflammatory and androgen-modulating mechanisms. The consistent increase in body weight across treatment groups

further indicates good tolerability and absence of overt toxicity. Overall, the data support the potential of *Acalypha indica* as a promising natural therapeutic candidate for managing benign prostatic hyperplasia and related androgen-mediated disorders.

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