

Scavenging Effects of *Annona Muricata* (Soursop) Leaf Extract Against Testosterone – Induced Prostate Hyperplasia in a Rat Model

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ABSTRACT

This study aimed to investigate the scavenging effects of *Annona Muricata* (soursop) leaf extract against testosterone – induced prostate hyperplasia in a rat model. A total of 78 adult male rats obtained from the laboratory animal production unit of the Department of Physiology, Abia State University, Uturu were used for the different segments of the study. Eighteen of the rats were used for acute toxicity/lethal dose evaluation of the extract while the remaining sixty (60) were used for the testosterone-induced hyperplasia study. Prostate hyperplasia was induced in the rats via daily sub-cutaneous administration of testosterone propionate (5 mg/kg body weight) for 28 days. Sixty rats used for this segment of the study were assigned to 6 groups of 10 rats and treated as follows: Group 1, Normal control, no treatment. Group 2 Testosterone propionate (5 mg/kg) only and was the BPH control. Group 3, Testosterone propionate (5 mg/kg body weight) + Finasteride (3 mg/kg body weight). Group 4, Testosterone propionate (5 mg/kg body weight) + *Annona Muricata* Leaf extract (300 mg/kg body weight). Group 5, Testosterone propionate (5 mg/kg bw) + *Annona Muricata* Leaf extract (600 mg/kg body weight). Group 6, *Annona Muricata* Leaf extract (600 mg/kg bw) only. At the end of treatment, animals were sacrificed and blood samples were collected into EDTA and plain bottles for determination of enzymatic antioxidants on serum samples for superoxide dismutase (SOD), catalase, glutathione peroxidase, was determined while reduced glutathione and Malondialdehyde (MDA) was determined spectrophotometrically. The Statistical Package for Social Sciences (SPSS, Version 20.0, IBM SPSS Inc., Chicago, IL) was used to analyze the collected data. One-way analysis of variance (ANOVA) was used to determine the level of significance. Data were analyzed using Duncan Multiple Range Test and complemented with Student's *t* test for post-hoc test for comparisons of the means of the various doses and fractions. *P*- values less than 0.05 were considered statistically significantly different between the test and control groups as well as among test groups for measured value. From this present study, *Annona muricata* (soursop) leaf extract displayed a diverse phytochemical profile rich in vitamins and antioxidants, which likely contributed to its observed antioxidant properties and ability to mitigate oxidative stress. These antioxidative effects were crucial in preserving cellular integrity and mitigating pathological changes induced by testosterone.

Keywords: *Annona muricata* (Soursop), scavenging effects, superoxide dismutase (SOD), catalase, glutathione peroxidase, reduced glutathione, malondialdehyde (MDA), testosterone – induced prostate hyperplasia, rat model

INTRODUCTION

The antioxidant properties of saponins contribute to their ability to protect cells from oxidative stress and damage. Saponins can scavenge free radicals and enhance the body's antioxidant defense mechanisms. This antioxidant activity is crucial in preventing cellular damage and reducing the risk of chronic diseases associated with oxidative stress, such as cardiovascular diseases and cancer [9,15,24]

A comprehensive analysis by Moghadamtousi *et al.* (2015)[17] revealed that the leaves of *Annona muricata* are particularly rich in flavonoids, tannins, and phenolic acids. Key phenolic acids such as gallic acid, chlorogenic acid, and caffeic acid have been isolated, which exhibit potent antioxidant activities. Additionally, the presence of flavonoids like quercetin, rutin, and kaempferol contributes to the plant's bioactive profile. Phenolic compounds in *Annona muricata* play a crucial role in its antioxidant activity. These compounds can neutralize free radicals and reactive oxygen species (ROS), thereby preventing cellular damage and oxidative stress. The antioxidant capacity of phenolics is primarily attributed to their ability to donate hydrogen atoms or electrons and chelate metal ions [22]. For instance, quercetin and rutin are well-known for their high antioxidant potential, which can protect cells from oxidative damage [16]. *Annona muricata* contains a wide range of bioactive compounds, including essential oils, which are a key component of its phytochemical composition [20,25]. Essential oils are volatile aromatic compounds extracted from various parts of plants, including leaves, flowers, stems, and fruits. According to Bakkali *et al.*

(2008), these oils are well-known for their many medicinal qualities, which include antibacterial, anti-inflammatory, antioxidant, and anticancer effects.[7]

MATERIALS AND METHODS

Materials

Equipments used include sensitive weighing balance, Electric weighing balance, Manual blender, Dissecting set, Dissecting board, 5ml syringes, 1ml syringes, test tubes, serum bottles, Sample bottles, EDTA bottles, whatman No 1 filter paper, Measuring cylinders, Pasteur pipette, Cotton wool, Micro pipette, Rat cages, Feeding and water trough, Oral gavage, Glass column, Microscope, Glass TLC plates, microscope slides, Glass wool, funnel, Spatula, Retort stand, Hand gloves, TLC tank, beaker, Oven, freezer, Refrigerator, Spectrophotometer(722N, Mindray, MC Quijing, China), Centrifuge, Rotary evaporator, Soxhlet extractor, GC-MS machine, Chemoilluminescence machine and Haematology analyser (BC-2300, Mindray, MC Quijing, China).

ETHICAL APPROVAL

Ethical approval was obtained from research and ethical committee of the Department of Physiology, Abia State University, Uturu.

Animals

A total of 78 adult male rats obtained from the laboratory animal production unit of the Department of Biochemistry, Abia State University, Uturu were used for the different segments of the study. Eighteen of the rats were used for acute toxicity/lethal dose evaluation of the extract while the remaining sixty(60) were used for the testosterone-induced hyperplasia study. The animals were housed in aluminum cages, under standard conditions 12/12h light-dark cycle at temperature of 25±2°C and

were fed with standard grower pellets (Grand Cereals Ltd, Abia State) and had access to clean drinking water for seven days of acclimatization before the initiation of experiment. All experiments were carried out in accordance with International Guideline for Biomedical Research (CIOMS/OMS, 1985) and as approved by the Department of Physiology, Abia State University, Uturu.

Experimental Design for the Hyperplasia Study

Prostate hyperplasia was induced in the rats via daily sub-cutaneous administration of testosterone propionate (5 mg/kg body weight) for 28 days. Sixty rats used for this segment of the study were assigned to 6 groups of 10 rats and treated as follows:

- | | | |
|---------|---|---|
| Group 1 | - | Normal control, no treatment |
| Group 2 | - | Testosterone propionate (5 mg/kg) only and was the BPH control |
| Group 3 | - | Testosterone propionate (5 mg/kg bw) + Finasteride (3 mg/kg body weight) |
| Group 4 | - | Testosterone propionate (5 mg/kg body weight) + AML extract (300 mg/kg bw) |
| Group 5 | - | Testosterone propionate (5 mg/kg body weight) + AML extract (600 mg/kg body weight) |
| Group 6 | - | AML extract (600 mg/kg body weight) only |

At the end of treatment, animals were sacrificed, and blood samples were collected into EDTA and plain bottles for biochemical analysis respectively. For the later (blood contained in plain bottles), the blood was allowed to clot and retract within two hours and then centrifuged at 3000 rpm for 10 minutes to obtain clear sera samples which were used for the various biochemical analyses.

Preparation of Plant Extracts

Plant extract were prepared in accordance with the method used by Orieki *et al.*, (2019). The freshly collected leaves of *Annona muricata* were air dried under shade for within 14 days and were thereafter pulverized into coarse powdered form using a locally fabricated grinding machine. Two solvents, ethanol and N-hexane were used for the preparation of the extracts. A known quantity (300g) of the powdered plant material was macerated in 1.5 litres of ethanol (AR) for 48 hours, been vigorously shaken every 2 hours within the period and was thereafter filtered with Whatman No. 1 filter paper to obtained a filtrate which was the extract in solution. The filtrate so obtained was concentrated at low temperature to obtain a solid dark-green extract which weighed 13.91 g and represented 4.64% extract yield. The extract was preserved in the refrigerator at -4 °C until needed and is hereafter referred to as *Annona muricata* leaf (AML) extract.

Determination of Enzymatic Antioxidants on Serum Samples Estimation of superoxide dismutase (SOD)

Superoxide dismutase activity was assayed by the method of Arthur and Boyne (1985) as contained in Randox kit.

Principle: Xanthine and xanthine oxidase are used in the process to produce superoxide radicals, which then react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T.) to produce a red formazan dye. The degree of inhibition of this reaction is then used to calculate the superoxide dismutase activity. Under assay circumstances, a 50% inhibition rate in the decrease of I.N.T. is caused by one unit of SOD.

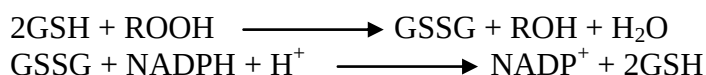
Procedure: After 30 seconds, the initial absorbance was measured after adding 1.7

ml of mixed substrate solution and 0.25 ml of mixed xanthine oxidase to 0.05 ml of diluted sample in a test tube. After three minutes, the final absorbance was measured, and SOD units per gram of hemoglobin were calculated using a standard curve.

Estimation of Catalase

The activity of catalase was assayed by the method of Sinha (1972).

Principle: When heated in the presence of hydrogen peroxide, dichromate in acetic acid was converted to chromic acetates, with peroxide forming as an unstable intermediate. At 570 nm, the amount of chronic acetate that was produced was measured. Catalase was given varying amounts of time to split H₂O₂. Chromic acetate was measured colorimetrically to assess the amount of H₂O₂ that remained after the reaction was stopped at various intervals by adding a dichromate acetic acid mixture in a 1:3 ratio.



Procedure: A known volume, 0.05ml of heparinised whole blood was diluted with 2ml of diluting reagent and this was used for the assay. A volume, 50ml of dilute sample was mixed with 1ml of phosphate buffer + EDTA, glutathione + glutathione reductase + NADPH and cumene hydroperoxide respectively. The timer began at the same moment as the beginning absorbance of the test and blank was measured once per minute. At 340 nm, the absorbances were measured once more after one and two minutes. The glutathione peroxidase activity was calculated as follows:

U/L of haemolysate = $8H_{12} \times DA_{340\text{nm}} / \text{minute}$.

Non-enzymatic antioxidants

Procedure: Two milliliters of H₂O₂ and two milliliters of phosphate buffer were added to 0.9 milliliters of distilled water and 0.1 milliliters of plasma in a test tube. Two milliliters of dichromate acetic acid reagent were added to one milliliter of this mixture to start the reaction. For two minutes, the absorbance of the reaction was measured at 30-second intervals. The unit of measurement for catalase activity was U/ml of plasma, or U-micromoles of H₂O₂ used per second.

Estimation of Glutathione Peroxidase

This was done according to the method of Paglia and Valentine (1967).

Principle: Glutathione (GPx) catalyses the oxidation of reduced glutathione (GSH) by cumene hydroperoxide. In the presence of glutathione reductase (GR) and NADPH, the oxidized glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH and NADP⁺. The decrease in absorbance at 340nm is measured.

Estimation of Reduced Glutathione

The Exner et al. (2000) method was used to calculate the reduced glutathione concentration.

Principle: This technique is based on the fact that adding 5, 5-dithio-bis-2-nitrobenzoic acid (DTNB) to a molecule that contains sulphhydryl groups causes it to turn yellow. A spectrophotometer was used to measure the color generated at 412 nm. The requirements are as follows:

1. 0.3M disodium salt of EDTA
2. 0.1% disodium salt of EDTA
3. **Precipitating agent:** In one liter of distilled water, combine 1.67 grams of metaphosphoric acid, 0.2 grams of

- EDTA disodium salt, and 30 grams of sodium chloride.
4. **5, 5-dithio-bis-2-nitrobenzoic (DTNB) reagent:** 100 milliliters of 1% sodium citrate with 40 milligrams of DTNB.
 5. **Standard solution:** 10mg of reduced glutathione in 100ml distilled water.

Procedure: 1.8 milliliters of EDTA solution were combined with 0.2 milliliters of the sample. Before centrifugation, 3.0 ml of precipitating reagent was added, properly mixed, and left for 5 minutes. 4 ml of 0.3 m disodium hydrogen phosphate solution and 1 ml of DTNB reagent were added to 2 ml of the supernatant, and the color generated was measured in a spectrophotometer at 412 nm. Similar treatment was given to a series of standard solutions that included 20–100 mg of reduced glutathione. For plasma, the data were given as mg/dl.

Malondialdehyde (MDA) Determination

Malondialdehyde (MDA) concentration was determined spectrophotometrically according to the method described by Atasaya *et al.*, (2004).

Test Principle: MDA reacts with thiobarbituric acid (TBA) to form a red or pink coloured complex which in acid solution absorbs maximally at 532nm. Liquid degradation occurs forming such products as malondialdehyde (from fatty acids with two or more double bonds),

ethane and pentane (from the n-terminal carbons of 3 and 6 fatty acids respectively).

Procedure: A volume 0.1 ml of serum was mixed with 0.9ml of water in a beaker. After that, 0.5ml of 25% trichloroacetic acid (TCA) and 0.5ml of 1% TBA (thiobarbituric acid) in 0.3%, NaOH were also added to the mixture. In a water bath, the mixture was cooked for 40 minutes at 95 degrees Celsius before being cooled in cold water. The cooled solution was then thoroughly mixed with 0.1 ml of 20% sodium dodecyl sulfate (SDS). In comparison to the blank, the absorbance was measured at 532 nm and 600 nm.

$$\% \text{ MDA} = \frac{A_{532} - A_{600}}{0.5271 \times 0.1} \times \frac{100}{1} \text{ (mg/dl)}$$

Data Analysis

Statistical Package for Social Sciences (SPSS, Version 20.0, IBM SPSS Inc, Chicago, IL) was used for data analysis. Level of significance was calculated by One Way Analysis of Variance (ANOVA). Data were analyzed using Duncan Multiple Range Test and complemented with Student's t test for post-hoc test for comparisons of the means of the various doses and fractions. P- values less than 0.05 were considered statistically significantly different between the test and control groups as well as among test groups for measured value.

RESULTS

Table 1: Qualitative phytochemical composition of *annona muricata* (soursop) leaf extract.

Phytochemical	Qualitative results
Saponins	++
Steroids	+
Flavonoids	++
Phenolics	++
Terpenoids	+
Glycosides	+

Alkaloids	++
Tannins	+
Triterpenes	-
Phlobatanins	-

Keywords: ++ = moderate concentration

+ = low concentrations.

The result of the qualitative phytochemical composition of *Annona muricata* leaf extract, as presented in Table 1, reveals that saponins, flavonoids, phenolics, and alkaloids are present in moderate

concentrations (++) while steroids, terpenoids, glycosides, and tannins are present in low concentrations (+). Triterpenes and phlobatanins are absent (-).

Table 2: Quantitative phytochemical contents of *Annona muricata* leaf extract.

Phytochemicals	Quantitative availability (mg/100g)
Saponins	11.11±0.61
Steroids	6.38±0.05
Flavonoids	24.42±0.82
Phenolics	19.20±0.32
Terpenoids	4.90±0.07
Glycosides	6.01±0.10
Alkaloids	38.35±0.38
Tannins	8.47±0.13

Values are presented as mean ± standard deviation (n = 3).

The result of the quantitative phytochemical contents of *Annona muricata* leaf extract, as presented in Table 4.2, shows that alkaloids are the most abundant phytochemical (38.35±0.38 mg/100g), followed by flavonoids (24.42±0.82 mg/100g) and phenolics

(19.20±0.32 mg/100g). Saponins (11.11±0.61 mg/100g) and tannins (8.47±0.13 mg/100g) are also present in significant amounts. Steroids (6.38±0.05 mg/100g), glycosides (6.01±0.10 mg/100g), and terpenoids (4.90±0.07 mg/100g) are present in lower quantities.

Table 3: Vitamins and minerals available concentration in *Annona muricata* leaf extract.

Vitamins/minerals	Available concentration (mg/100g)
Iron	12.63±0.66
Vitamin B ₁	4.44±0.13
Vitamin B ₂	6.83±0.16
Vitamin B ₆	4.44±0.08
Vitamin B ₁₂	15.57±0.07
Vitamin C	35.50±0.26
Vitamin E	23.28±0.45

Values are presented as mean ± standard deviation (n = 3).

The result of the available concentration of vitamins and minerals in *Annona muricata* leaf extract, as presented in Table 3, shows

that Vitamin C has the highest concentration (35.50±0.26 mg/100g. Vitamins B₂ (6.83±0.16 mg/100g), B₁

(4.44 ± 0.13 mg/100g), and B6 (4.44 ± 0.08 mg/100g) are present in lower quantities.

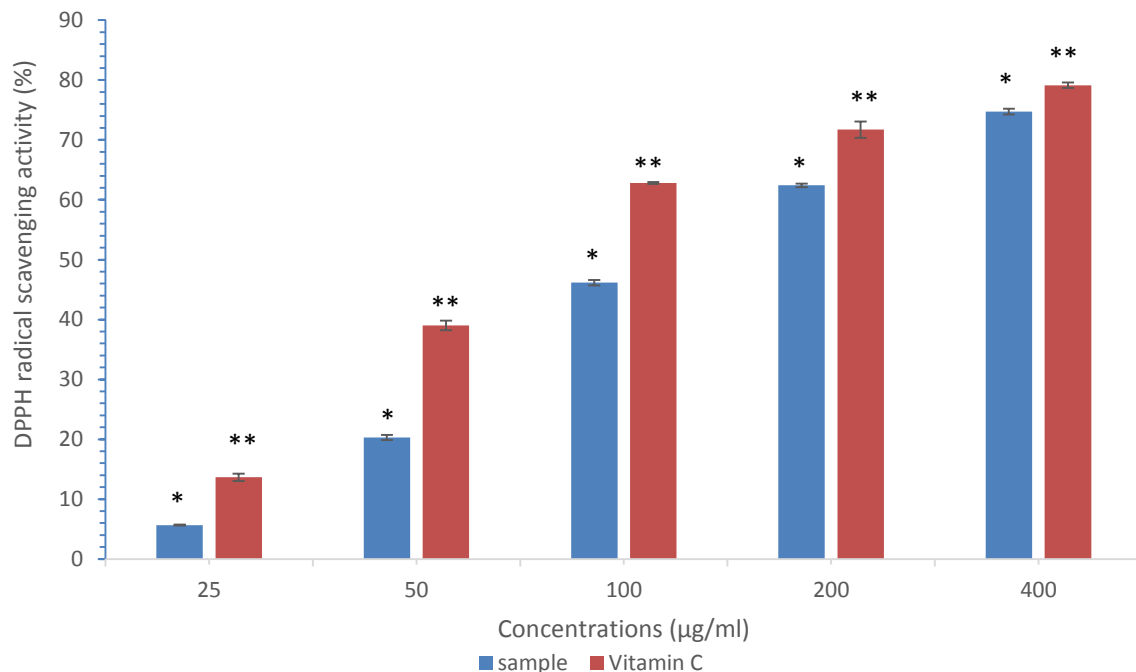


Fig. 1: DPPH radical scavenging activity of *annona muricata* leaf extract.

Bars are presented as mean $\pm$ standard deviation (n = 3); and bars with double asterisk (**) are significantly (P < 0.05) different from paired bar with a single asterisk (*).

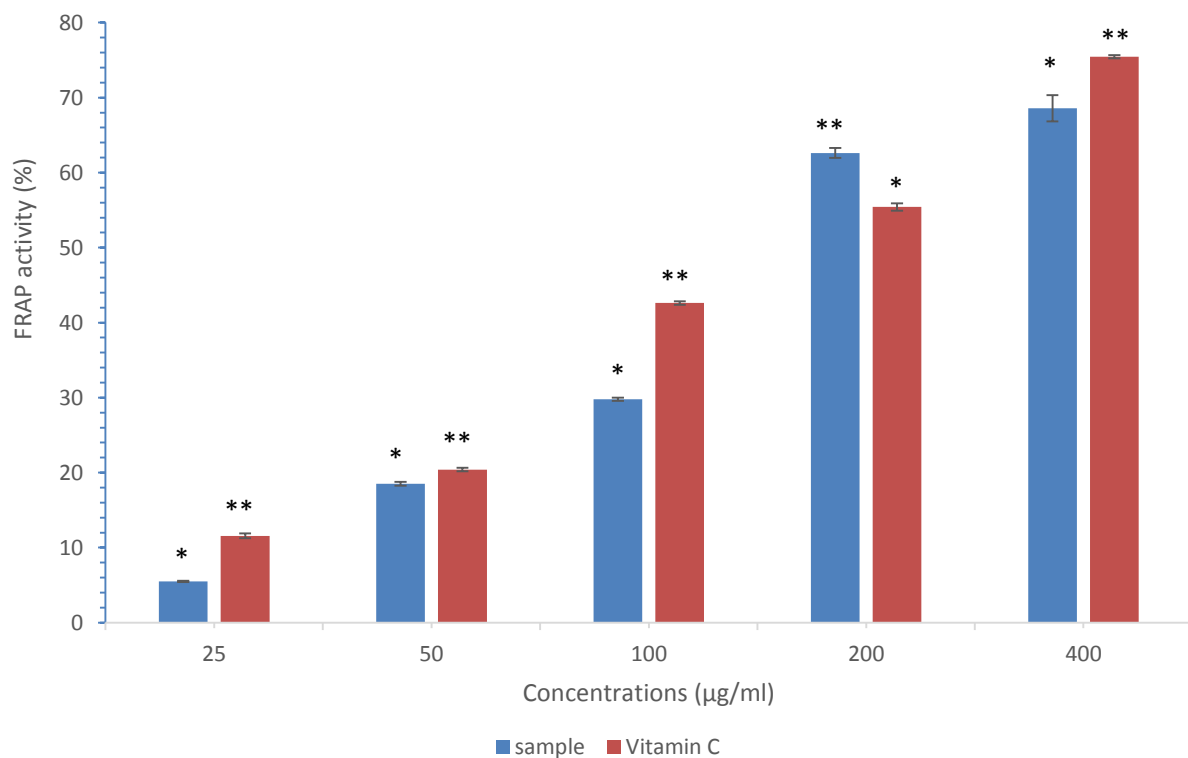


Fig. 2: Ferric reducing antioxidant power (FRAP) of *annona muricata* leaf extract.

Bars are presented as mean $\pm$ standard deviation (n = 3); and bars with double asterisk (**) are significantly (P < 0.05) different from paired bar with a single asterisk (*).

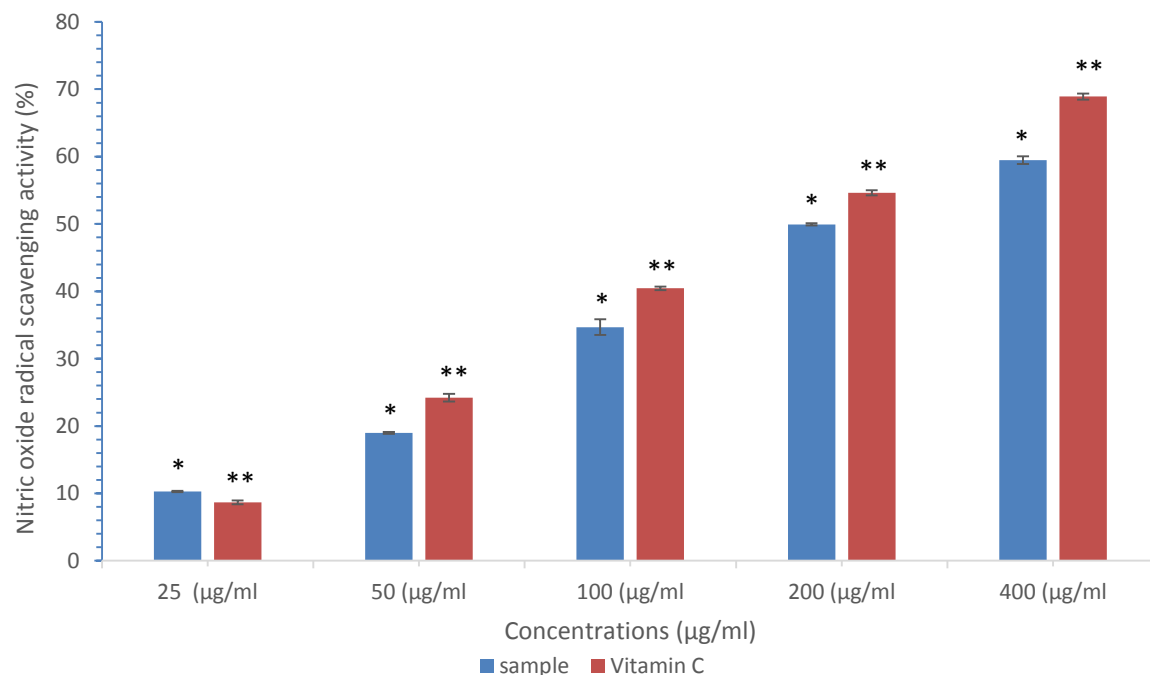


Fig. 3: Nitric oxide scavenging activity of *annona muricata* in testosterone – induced prostate hyperplasia.

Bars are presented as mean $\pm$ standard deviation (n = 3); and bars with double asterisk (**) are significantly (P < 0.05) different from paired bar with a single asterisk (*).

Table 4: Antioxidants scavenging activities of *annona muricata* (soursop) leaf extract in BPH.

Treat ments	Norm al contr ol	BPH control (Testosteron e only)	Finesteride (3 mg/kg) + Testosterone	AML extract (200 mg/kg) + Testosterone	AML extract (400 mg/kg) + Testosterone	AML extract (800 mg/kg) + Testosterone
GSH (mg/d l)	13.27 $\pm$ 0.87 _{b,c}	11.40 $\pm$ 0.72 ^a	12.17 $\pm$ 0.55 ^{a,b}	13.57 $\pm$ 0.81 ^c	13.73 $\pm$ 0.45 ^c	14.37 $\pm$ 0.21 ^c
GPx (u/l)	46.57 $\pm$ 1.16 _{c,d}	36.37 $\pm$ 2.75 ^a	41.90 $\pm$ 1.76 ^b	43.03 $\pm$ 2.04 ^b	44.73 $\pm$ 1.16 ^{b,c}	48.27 $\pm$ 0.35 ^d
SOD (u/l)	29.50 $\pm$ 1.65 _{b,c}	25.63 $\pm$ 1.27 ^a	25.93 $\pm$ 0.67 ^{a,b}	28.37 $\pm$ 1.01 ^c	27.83 $\pm$ 0.60 ^{b,c}	30.87 $\pm$ 1.22 ^d
CAT (u/l)	23.03 $\pm$ 1.17 _b	19.57 $\pm$ 1.24 ^a	19.63 $\pm$ 0.95 ^a	22.17 $\pm$ 1.01 ^{a,b}	23.53 $\pm$ 0.97 ^b	23.13 $\pm$ 2.45 ^b
MDA (mmo l/L)	0.28 $\pm$ 0.01 ^{a,b}	0.48 $\pm$ 0.04 ^e	0.38 $\pm$ 0.02 ^d	0.34 $\pm$ 0.04 ^{c,d}	0.32 $\pm$ 0.03 ^{b,c}	0.26 $\pm$ 0.02 ^a

Values are presented as mean $\pm$ standard deviation ($n = 3$); and means with different letter superscripts are significantly ($P < 0.05$) different from paired mean across the row.

The result of antioxidant levels as presented in Table 4.4 reveals that the levels of GSH, GPx, SOD, CAT, and MDA are significantly affected by the treatments. The normal control group shows moderate levels of GSH (13.27 ± 0.87 mg/dl), GPx (46.57 ± 1.16 u/l), SOD (29.50 ± 1.65 u/l), and CAT (23.03 ± 1.17 u/l), with a low level of MDA (0.28 ± 0.01 mmol/L). The BPH control group (Testosterone only) has significantly reduced levels of GSH (11.40 ± 0.72 mg/dl), GPx (36.37 ± 2.75 u/l), SOD

(25.63 ± 1.27 u/l), and CAT (19.57 ± 1.24 u/l), and an elevated level of MDA (0.48 ± 0.04 mmol/L). Treatment with Finasteride (3 mg/kg) + Testosterone increases GSH (12.17 ± 0.55 mg/dl), GPx (41.90 ± 1.76 u/l), SOD (25.93 ± 0.67 u/l), and CAT (19.63 ± 0.95 u/l), and decreases MDA (0.38 ± 0.02 mmol/L) compared to the BPH control. AML extract at doses of 200 mg/kg, 400 mg/kg, and 800 mg/kg + Testosterone significantly increases GSH, GPx, SOD, and CAT levels, and decreases MDA levels, with the 800 mg/kg dose showing the highest antioxidant levels and the lowest MDA (0.26 ± 0.02 mmol/L). These results indicate that AML extract improves antioxidant status in a dose-dependent manner.

Table 5: Body Weight Changes.

Treatment groups	Initial body weight (g)	Final body weight (g)	Weight gain (g)	Percentage weight gain (%)
Normal control	81.27 ± 3.84^a	170.10 ± 11.51^a	88.83 ± 7.69^a	109.18 ± 4.29^b
BPH control (Testosterone only)	105.02 ± 1.24^c	191.17 ± 25.52^a	86.16 ± 26.03^a	$82.13 \pm 25.24^{a,b}$
Finasteride (3 mg/kg) + Testosterone	120.34 ± 11.90^d	195.88 ± 12.78^a	75.54 ± 1.90^a	63.15 ± 5.81^a
AML extract (200 mg/kg) + Testosterone	$90.55 \pm 9.48^{a,b}$	170.85 ± 16.58^a	80.30 ± 8.45^a	$88.86 \pm 6.70^{a,b}$
AML extract (400 mg/kg) + Testosterone	$90.60 \pm 8.54^{a,b}$	179.51 ± 11.72^a	88.91 ± 18.66^a	$99.90 \pm 30.10^{a,b}$
AML extract (800 mg/kg) + Testosterone	$95.84 \pm 0.97^{b,c}$	181.96 ± 20.34^a	86.12 ± 19.37^a	$89.73 \pm 19.41^{a,b}$

Values are presented as mean $\pm$ standard deviation ($n = 3$); and means with different letter superscripts are significantly ($P < 0.05$) different from paired mean within the column.

The result of body weight changes as presented in Table 4.5 shows that the Normal control group had a significant weight gain of 88.83 ± 7.69 g, with a percentage weight gain of $109.18 \pm 4.29\%$. The BPH control group (Testosterone only) had a weight gain of 86.16 ± 26.03 g,

with a percentage weight gain of $82.13 \pm 25.24\%$. The Finasteride (3 mg/kg) + Testosterone group showed the lowest weight gain of 75.54 ± 1.90 g and the lowest percentage weight gain of $63.15 \pm 5.81\%$. The AML extract (400 mg/kg) + Testosterone group had the highest weight gain of 88.91 ± 18.66 g and a percentage weight gain of $99.90 \pm 30.10\%$. All treatment groups showed no significant differences in final body weight compared to the initial body weight, indicating no

major weight loss or gain across treatments.

DISCUSSION

The analysis of vitamins in *Annona muricata* leaf extract reveals a notable presence of Vitamin C, which is documented at a concentration of 35.50 ± 0.26 mg/100g. Vitamin C, known for its antioxidant properties, plays a crucial role in scavenging free radicals and protecting cells from oxidative stress-induced damage. This aligns with studies demonstrating the antioxidant effects of *Annona muricata* extract, which could be attributed in part to the presence of Vitamin C [12,21]. Vitamins B2 (riboflavin), B1 (thiamine), and B6 (pyridoxine) are present in lower quantities in the extract: 6.83 ± 0.16 mg/100g, 4.44 ± 0.13 mg/100g, and 4.44 ± 0.08 mg/100g, respectively. These B-complex vitamins are essential for various metabolic processes in the body, including energy production and nerve function (Offor *et al.*, 2015). While their concentrations are lower compared to Vitamin C, their presence underscores the nutritional value of *Annona muricata* extract, contributing to its potential health benefits beyond antioxidant activity. The presence of these vitamins complements the extract's phytochemical profile, enhancing its overall therapeutic potential. Studies have highlighted the multifaceted pharmacological effects of *Annona muricata* extract, including hepatoprotective, nephroprotective, and anti-inflammatory properties, which are likely supported by the synergistic action of vitamins and phytochemicals present in the extract [3,10,13].

The antioxidant properties of *Annona muricata* leaf (AML) extract have been extensively studied, revealing significant effects on various antioxidant markers as observed in the experimental setups [4,12,18]. In the context of oxidative

stress-induced conditions like benign prostatic hyperplasia (BPH), the antioxidant capacity of AML extract becomes particularly relevant. In the study, it was found that the normal control group exhibited moderate levels of key antioxidants: reduced glutathione (GSH) at 13.27 ± 0.87 mg/dl, glutathione peroxidase (GPx) at 46.57 ± 1.16 u/l, superoxide dismutase (SOD) at 29.50 ± 1.65 u/l, catalase (CAT) at 23.03 ± 1.17 u/l, and a low level of malondialdehyde (MDA) at 0.28 ± 0.01 mmol/L, indicating balanced antioxidant status (Ajayi *et al.*, 2024; Handayani *et al.*, 2022). Conversely, the BPH control group induced by testosterone showed significantly reduced levels of GSH, GPx, SOD, and CAT, along with elevated MDA levels, indicative of heightened oxidative stress and impaired antioxidant defense mechanisms [27]. Treatment with Finasteride, a reference drug for BPH, in combination with testosterone, partially restored antioxidant levels, albeit not to the extent observed with AML extract treatments [1]. Specifically, Finasteride + Testosterone treatment increased GSH, GPx, SOD, and CAT levels, while decreasing MDA levels compared to the BPH control group. AML extract treatments at doses of 200 mg/kg, 400 mg/kg, and 800 mg/kg + Testosterone demonstrated a dose-dependent improvement in antioxidant status. With the strongest benefits occurring at the highest dosages (400 mg/kg and 800 mg/kg), these therapies markedly raised GSH, GPx, SOD, and CAT levels while lowering MDA levels. The 800 mg/kg dose particularly stood out, exhibiting the highest antioxidant levels and the lowest MDA level (0.26 ± 0.02 mmol/L), indicating potent antioxidant activity [5,12]. These findings underscore the potent antioxidant potential of *Annona muricata* leaf extract, attributed to its rich phytochemical composition including flavonoids, alkaloids, and phenolics. The extract's potential as a treatment for

oxidative stress-related conditions, such as BPH, is demonstrated by its capacity to increase antioxidant enzyme activities and lower lipid peroxidation. These results support the traditional use of *Annona muricata* in natural medicine and suggest its potential as a novel therapeutic agent for oxidative stress-associated conditions.

The assessment of body weight changes in experimental groups treated with *Annona muricata* leaf (AML) extract provides insights into its physiological effects, particularly in the context of conditions like benign prostatic hyperplasia (BPH) induced by testosterone [6,8]. In the study, the normal control group exhibited a significant weight gain of $88.83 \pm 7.69\text{g}$, corresponding to a percentage weight gain of $109.18 \pm 4.29\%$. This increase reflects normal growth and health maintenance in the absence of any pathological condition or treatment [14,23]. Conversely, the BPH control group, induced with testosterone, showed a lower weight gain of $86.16 \pm 26.03\text{g}$, with a percentage weight gain of $82.13 \pm 25.24\%$. This slight decrease in weight gain compared to the normal control group might indicate the metabolic impact of testosterone-induced BPH and associated physiological changes [2]. Treatment with Finasteride, a standard drug for BPH management, in combination with testosterone, resulted in the lowest weight gain of $75.54 \pm 1.90\text{g}$ and the lowest percentage weight gain of $63.15 \pm 5.81\%$. This observation aligns with the known side effects of Finasteride on body weight regulation [26]. In contrast, treatment with AML extract at 400 mg/kg + Testosterone demonstrated the highest weight gain of $88.91 \pm 18.66\text{g}$ and a percentage weight gain of $99.90 \pm 30.10\%$. Despite the higher weight gain compared to Finasteride, it did not significantly differ from the normal control group, indicating that AML extract treatment did not adversely affect body weight and possibly contributed positively to overall health and

metabolic balance [11,19]. These results suggest that AML extract, particularly at the studied dose, does not induce adverse effects on body weight and may even support metabolic stability. This finding reinforces the potential of AML extract as a safe adjunctive therapy in managing conditions like BPH, offering a natural alternative with potential health benefits beyond symptom management.

CONCLUSION

From this present study, *Annona muricata* (soursop) leaf extract displayed a diverse phytochemical profile rich in vitamins and antioxidants, which likely contributed to its observed antioxidant properties and ability to mitigate oxidative stress. These antioxidative effects were crucial in preserving cellular integrity and mitigating pathological changes induced by testosterone

REFERENCES

1. Abdul Wahab, S. M., Jantan, I., Haque, M. A., & Arshad, L. (2018). Exploring the leaves of *Annona muricata* L. as a source of potential anti-inflammatory and anticancer agents. *Frontiers in Pharmacology*, 9, 344514. <https://doi.org/10.3389/fphar.2018.00344>
2. Adewole, S. O., & Ojewole, J. A. O. (2006). Protective effects of *Annona muricata* Linn. (Annonaceae) leaf aqueous extract on serum lipid profiles and oxidative stress in hepatocytes of streptozotocin-treated diabetic rats. *African Journal of Traditional, Complementary and Alternative Medicines*, 3(1), 39-52.
3. Ajayi, A. A., Ajayi, B. A., & Ajayi, T. F. (2024). Assessing the impact of *Annona muricata* aqueous extract on liver enzymes in Wistar rats exposed to paracetamol overdose. *International Journal of Biochemistry Research and Review*, 33(5), 91-96.

- <https://doi.org/10.9734/ijbcurr/2024/v33i530139>
4. Al-Medhtiy, M. H., Jabbar, A. A., Shareef, S. H., Ibrahim, I. A. A., Alzahrani, A. R., & Abdulla, M. A. (2022). Histopathological evaluation of *Annona muricata* in TAA-induced liver injury in rats. *Processes*, 10(8), 1613.
<https://doi.org/10.3390/pr10081613>
 5. Arthur, F., Woode, E., Terlabi, E., & Larbie, C. (2012). Evaluation of hepatoprotective effect of aqueous extract of *Annona muricata* (Linn.) leaf against carbon tetrachloride and acetaminophen-induced liver damage. *Journal of Natural Pharmaceuticals*, 3(1), 25-25.
<https://doi.org/10.4103/2229-3301.97806>
 6. Asare, G. A., Afriyie, D., Ngala, R. A., Abutiate, H., Doku, D., Mahmood, S. A., & Rahman, H. (2015). Antiproliferative activity of aqueous leaf extract of *Annona muricata* L. on the prostate, BPH-1 cells, and some target genes. *Integrative Cancer Therapies*, 14(1), 65-74.
<https://doi.org/10.1177/1534735414553187>
 7. Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils—a review. *Food and Chemical Toxicology*, 46(2), 446-475.
<https://doi.org/10.1016/j.fct.2007.09.106>
 8. Bernardine, O. O., Peter, A. U., & Bernard, K. N. (2024). Histopathological and biochemical assessment of *Annona muricata* and *Allium sativum* pooled extract on testosterone-induced prostate hyperplasia in albino rats. *Journal of Complementary and Alternative Medical Research*, 25(4), 21-30.
 9. Chen, I. C., Chen, M. T., & Chung, T. W. (2020, December). Analysis of antioxidant property of the extract of saponin by experiment design methodology. In *IOP Conference Series: Earth and Environmental Science*, 594(1), 012002. IOP Publishing.
<https://doi.org/10.1088/1755-1315/594/1/012002>
 10. Cho, E. J., Lee, J. H., Sung, N. Y., & Byun, E. H. (2017). Anti-inflammatory effects of *Annona muricata* leaf ethanol extracts. *Journal of the Korean Society of Food Science and Nutrition*, 46(6), 681-687.
<https://doi.org/10.3746/jkfn.2017.46.6.681>
 11. Elmas, O., Sahin, H. H. K., Guven, B., Abuzaid, M. M., Elshami, W., AlMisned, G., ... & Tekin, H. O. (2022). A focusing study on radioprotective and antioxidant effects of *Annona muricata* leaf extract in the circulation and liver tissue: Clinical and experimental studies. *Open Chemistry*, 20(1), 920-928.
<https://doi.org/10.1515/chem-2022-0244>
 12. Gavamukulya, Y., Wamunyokoli, F., & El-Shemy, H. A. (2017). *Annona muricata*: Is the natural therapy to most disease conditions including cancer growing in our backyard? A systematic review of its research history and future prospects. *Asian Pacific Journal of Tropical Medicine*, 10(9), 835-848.
<https://doi.org/10.1016/j.apjtm.2017.07.023>
 13. Handayani, S. I., Sari, M. I. P., Sardjana, M. S., Kusmardi, K., Nurbaya, S., Rosmalena, R., ... & Prasasty, V. D. (2022). Ameliorative effects of *Annona muricata* leaf ethanol extract on renal morphology of alloxan-induced mice. *Applied Sciences*, 12(18), 9141.
<https://doi.org/10.3390/app12189141>
 14. Humphrey, P. A. (2017). Histopathology of prostate cancer. *Cold Spring Harbor Perspectives in*

- Medicine*, 7(10), a030411.
<https://doi.org/10.1101/cshperspect.a030411>
15. Khan, M. I., Karima, G., Khan, M. Z., Shin, J. H., & Kim, J. D. (2022). Therapeutic effects of saponins for the prevention and treatment of cancer by ameliorating inflammation and angiogenesis and inducing antioxidant and apoptotic effects in human cells. *International Journal of Molecular Sciences*, 23(18), 10665. <https://doi.org/10.3390/ijms231810665>
16. Manach, C., Scalbert, A., Morand, C., Rémésy, C., & Jiménez, L. (2004). Polyphenols: Food sources and bioavailability. *The American Journal of Clinical Nutrition*, 79(5), 727-747. <https://doi.org/10.1093/ajcn/79.5.727>
17. Moghadamtousi, S. Z., Fadaeinasab, M., Nikzad, S., Mohan, G., Ali, H. M., & Kadir, H. A. (2015). *Annona muricata* (Annonaceae): A review of its traditional uses, isolated acetogenins, and biological activities. *International Journal of Molecular Sciences*, 16(7), 15625-15658. <https://doi.org/10.3390/ijms160715625>
18. Offor, C. E., Uko, A. U., Alum, E. U., Ugwu, O. P. C., & Agbafor, K. N. (2015). Effect of ethanol leaf-extract of *Annona muricata* on liver enzymes of albino rats. *IOSR Journal of Nursing and Health Science*, 4(5), 99-103. <https://doi.org/10.9790/1959-045199103>
19. Ogbu, P. N., Ugota, E. O., Onwuka, R. U., Ogbu, I. M., & Aloke, C. (2020). Effect of acetogenin fraction of *Annona muricata* leaves on antioxidant status and some indices of benign prostatic hyperplasia in rats. *Redox Report*, 25(1), 80-86. <https://doi.org/10.1080/13510002.2020.1713300>
20. Oyedele, O., Taiwo, F. O., Ajayi, O. S., Ayinde, F., Oziegbe, M., & Oseghare, C. O. (2015). Biocidal and phytochemical analysis of leaf extracts of *Annona muricata* (Linn.). *International Journal of Science: Basic and Applied Research*, 24(7), 76-87.
21. Padma, P., Chansouria, J. P. N., & Khosa, R. L. (1999). Hepatoprotective activity of *Annona muricata* Linn. and *Polyalthia cerasoides* Bedd. *Ancient Science of Life*, 19(1-2), 7-10.
22. Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine*, 20(7), 933-956. [https://doi.org/10.1016/0891-5849\(95\)02228-9](https://doi.org/10.1016/0891-5849(95)02228-9)
23. Robert, G., Descazeaud, A., Nicolaïew, N., Terry, S., Sirab, N., Vacherot, F., ... & De La Taille, A. (2009). Inflammation in benign prostatic hyperplasia: A 282 patients' immunohistochemical analysis. *The Prostate*, 69(16), 1774-1780. <https://doi.org/10.1002/pros.20987>
24. Shi, J., Arunasalam, K., Yeung, D., Kakuda, Y., Mittal, G., & Jiang, Y. (2004). Saponins from edible legumes: Chemistry, processing, and health benefits. *Journal of Medicinal Food*, 7(1), 67-78. <https://doi.org/10.1089/109662004322984073>
25. Souza, D. O., dos Santos Sales, V., de Souza Rodrigues, C. K., de Oliveira, L. R., Lemos, I. C. S., de Araújo Delmondes, G., ... & Kerntopf, M. R. (2018). Phytochemical analysis and central effects of *Annona muricata* Linnaeus: Possible involvement of the GABAergic and monoaminergic systems. *Iranian Journal of Pharmaceutical Research: IJPR*, 17(4), 1306.
26. Yang, C., Gundala, S. R., Mukkavilli, R., Vangala, S., Reid, M. D., & Aneja, R. (2015). Synergistic interactions

among flavonoids and acetogenins in *Annona muricata* extract enhance its antitumor activity in prostate cancer cells. *Journal of Natural Products*, 78(8), 1753-1763.
<https://doi.org/10.1021/acs.jnatprod.5b00223>

27. Zeweil, M. M., Khafaga, A. F., Mahmoud, S. F., Wasef, L., Saleh, H., Elrehim, A. M. A., ... & Ghoneim, H. A. (2024). *Annona muricata* L. extract restores renal function, oxidative stress, immunohistochemical structure, and gene expression of TNF- α , IL- β 1, and CYP2E1 in the kidney of DMBA-intoxicated rats. *Frontiers in Pharmacology*, 15, 1348145.
<https://doi.org/10.3389/fphar.2024.1348145>