



Bioactive profile, Antioxidant potential, and Functional Attributes of *Annona muricata* L (soursop) Leaf Extract on Weight in rats induced with Benign Prostate Hyperplasia

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Abstract: The bioactive profile, antioxidant potential and functional attributes of *A. muricata* leaf extract on weight in rats induced with benign prostate hyperplasia was investigated using standard methods. Result obtained for bioactive profile screening of the leaf extract revealed important bioactive constituents such as alkaloids, flavonoids, terpenoids, tannins, and some other bioactive constituents at different concentrations. The leaf extract also exhibited moderate scavenging potentials against standard antioxidant agents tested using different methods. The leaf extract also exhibited the potential to promote weight recovery in rats induced with benign prostate hyperplasia when compared to those treated with drugs, and showed significant improvements over the positive control group. The beneficial effects of the extract could be attributed to the bioactive constituents identified. This study has revealed the bioactive profile, antioxidant potential, and functional attributes of *A. muricata* leaf extract in improving weight in rats induced with benign prostate hyperplasia.

Keywords: *A. muricata* L., bioactive profile, antioxidant potential, functional attributes and benign prostate hyperplasia.

Introduction

Humans have relied on medicinal plants since ages past through pertinent observations and monitoring (Kaliyaperumal *et al.*, 2013; Mehboob *et al.*, 2022). The Alma-Ata declaration of 1978 by World Health Organization (WHO) facilitated a renewed interest in medicinal plants. According to Sofowora *et al.* (2013), a medicinal plant is any plant used for the extraction of pure substances either for direct medicinal compounds, which can be used for the synthesis of useful drugs. It therefore means that any plant whose part, one or more organs contains substances that can be used for therapeutic purpose or which can be used for the synthesis of useful drugs can be termed a medicinal plant (Duke, 2020). Different authors have associated the therapeutic values of medicinal plants to the presence of chemical substances found in the plants, which produce definite physiological action against disease causing pathogens (Odika *et al.*, 2022). These chemical substances have been identified as phytochemicals (Duke, 2020). Phytochemicals are bioactive non-nutrient compounds found in plants which confer resistance against fungal, bacterial, and pesticide pathogens (Dougharim *et al.*, 2009; Kumar *et al.*, 2023). Digafe *et al.* (2024) revealed that several biologically active plant

products such as alkaloids, flavonoids, polyphenols, saponins, tannins, terpenes, and their derivatives are some of the phytochemicals that have been figured out over the line of human evolution. Further studies have revealed the curative and therapeutic property of these phytochemicals in humans (Misral *et al.*, 2024). WHO in 2021 noted that medicinal plants represent the classical therapeutic source in developing countries of Africa, Asia, and South American continents. Significant antioxidants have been recorded as phytochemicals that aid medicinal plants' efficacy against diseases. Most plants that are medicinal in nature contain wide variety of flavonoids, vitamins, phenols, and terpenoids free scavenging molecules (Marghich *et al.*, 2022), which possess the ability to scavenge free radical in human body (Digafe *et al.*, 2024). Agomuo *et al.* (2017) noted that natural compounds found in plants or their synthetic forms are the basis of modern pharmacopeia. Extracts, infusions, decoctions or concoctions made from medicinal plant are increasingly emerging as a substitute or complimentary therapy in the treatment of different diseases globally.

Benign prostate hyperplasia (BPH) is a urological condition associated with the enlargement and

proliferation of the prostate gland cells (Eleazu *et al.*, 2021). Kalu *et al.* (2016) described prostate gland as an accessory gland associated with the male reproductive system which surrounds the neck of urinary bladder and proximal portion of the urethra. BPH is a non-malignant enlargement and manifest clinically with symptoms at the lower urinary tract (Kevin *et al.*, 2024). The principles and molecular mechanisms behind the etiology as well as the pathogenesis of BPH have not fully been unraveled (Ugwu *et al.*, 2018). However, factors such as age related hormonal alterations, inflammatory mediators, environmental factors, dietary factors, and oxidative stress have been implicated in the etiology of BPH (Eleazu *et al.*, 2017).

A. muricata L. popularly known as soursop is one of the medicinal plants, which has attracted a renewed interest in line with Alma-Ata declaration and it is used in phytomedicine. It belongs to the family Annonaceae (Coria-Téllez *et al.*, 2018). The plant is a native to North and South America, and is widely distributed within the tropical as well as the subtropical parts of the world such as Nigeria, India, and Malaysia (Moghadamtousi *et al.*, 2015). *A. muricata* is a terrestrial evergreen and erect tree that grows up to 8 meter in height. It bears dark

green leaves and produces edible fruits, which are heart-shaped and can reach up to 20 cm in diameter (De Souza *et al.*, 2009; Moghadamtousi *et al.*, 2015). The folk medicinal importance of concoctions, infusions, syrups, and extracts made from *A. muricata* cannot be overstated. Different parts of the plant possess properties that are utilized in phytomedicine (Yang *et al.*, 2015). Reviewed research studies have reported the antiulcer, antidiabetic, antiprotozoal, antidiarrhea, antibacterial, antiviral, antihypertensive, and wound healing activities of the plant (Wahab *et al.*, 2018; Agu *et al.*, 2019; Mutakin *et al.*, 2022; Opara *et al.*, 2024). It has been reported that different parts of the plant possess different pharmacological activities (Paola Balderrama-Carmona *et al.*, 2020). The seed of the plant has potential against parasitic infections. The fruit is effective against diseases such as diarrhea, nervous disorders, and arthritis (Solanki *et al.*, 2020). The leaves are used in the treatment of headaches, insomnia, and cystitis (Wélé *et al.*, 2003). Adewole and Ojewole (2009) reported the protective effects of *A. muricata* aqueous leaf extract on the lipid profiles and oxidative stress in hepatocytes of streptozocin-treated diabetic rats. Yunivita *et al.* (2019) reported the effect of the leaf infusion extract of *A. muricata* on glucose absorption in the

intestinal cells membrane of Wistar rats. Guevara-Vásquez *et al.* (2021) reported decreases intestinal glucose absorption and improves glucose tolerance in normal and diabetic rats placed on *A. muricata* extract. The anticancer activity of *A. muricata* on breast (Rady *et al.*, 2018) and cervical cancers (Solanki *et al.*, 2020) has been reported. There is no doubt that the importance of *A. muricata* lies in its phytochemical and pharmacological properties as well as its ethnomedicinal uses (Solanki *et al.*, 2020). Gavamukulya *et al.* (2017) in a systemic review of *A. muricata*, addressed it as a natural therapy to most disease conditions including cancer growing in our backyard. In line with the renewed interest in plant-based medicine, this study aims to address the limited literature on the effects of *A. muricata* leaf extract on BPH. This study specifically evaluated the bioactive profile, antioxidant activity, and functional attributes of *A. muricata* ethanolic leaf extract on body weight of BPH-induced rats.

Materials and Method

Plant Material Collection, Preparation and Identification

Fresh leaves of *A. muricata* were collected from the Imo State University (IMSU) school farm at Lake Nwebere, Owerri, Imo State in

August, 2024. The collected leaves were identified and authenticated by a taxonomist, Prof. F. N. Mbagwu of the department of Plant Science and Biotechnology, IMSU, Owerri. A voucher specimen catalogued IMSU/AM/2024/57 was deposited in the herbarium unit of Faculty of Sciences, IMSU for reference purposes. The leaves were cleaned, air-dried and pulverized. The pulverized form was stored using polythene bags and was maintained at room temperature till needed for further studies.

Analysis of Bioactive Profile

Bioactive compounds screened were alkaloids, saponins, flavonoids, terpenoids, tannins, Phlobataninnis, phenolics, glycosides, and steroids. The screening test for alkaloids, tannins, and flavonoids were carried out with the methods as described by Trease and Evans (1983). The screening tests for saponins and glycosides were carried out using the methods described by Agomuo *et al.* (2002), while the qualitative tests for phenolic compounds, phlobataninins, terpenoids, and steroid were done using the methods described by Harbone (1973).

Preparation of Extract

Two hundred grams (200g) of the sample was measured using weighing balance (Metter,

Germany), and dissolved in 500 ml of 70% ethanol for 48 hours at room temperature which was intermittent stirred. The mixture obtained was filtered using Whatman No 1 filter paper into a beaker. The filtrate was further concentrated at the temperature of 40°C with the help of an electric oven. The percentage of the yield was calculated before the extract was stored in a refrigerator at 4°C.

$$\% \text{ yield} = \frac{\text{Weight of the extract}}{\text{Weight of sample measured}} \times 100/1$$

Estimation of Antioxidant Potential

The assessment of 2,2-diphenyl-2-picrylhydryl (DPPH) using the method as described in the literature published by Chekuri *et al.* (2018). Nitric oxide radical scavenging activity was determined using the method as described in the literature published by method of Marcocci *et al.* (1994). Lipid peroxidation scavenging activity was evaluated with the method as described by Chang and Kim (2018), while the reducing potential of the extract was evaluated using the method as described by Fejes *et al.* (2000).

Acute Toxicity Test

The up and down method of acute toxicity (OECD, 2008) was used for acute toxicity test. Six rats were selected randomly, weighed and

placed in a cage. Three of the rats were treated with 2 g/kg of the plant extract while the remaining three rats were given equal volume of distilled water through oral gastric gavage. The rats were observed for 48 hours for signs of toxicity and mortality. The acute toxicity of the leaf extract is more than 5000 mg/kg.

Experimental Animals and Research Design

A total of ninety-eight male albino rats (Wistar strain; weighing between 150-165) were obtained from the animal colony of Department of Biochemistry, Rhema University Nigeria, Aba. The rats were placed in a standard cage and were allowed to acclimatize to the conditions (temperature of (25 - 28°C), humidity (35 - 60%) and 12hr/12hr light/darkness cycle) of the environment in the animal house of the Department of Biochemistry, University of Agriculture and Environmental Sciences (UAES), Umuagwo, Imo State. They were placed on the same feed and water (potable water) for the initial acclimatization of two weeks.

After acclimatization, the rats were weighed and allocated to seven groups (I-VII) of 14 rats each. They were then induced with BPH using a mixture of olive oil and testosterone-propionate (4 mg/kg), which was injected

subcutaneously at different sites on the rats daily for 15 days. After 15 days, two rats from each group were anesthetized using chloroform inhalation and sacrificed by cardiac puncture. Their prostate glands were harvested, rinsed and weighed to ascertain enlargement. The remaining twelve (12) rats in each group were used for further studies.

The treatments given to the groups are as follows.

Group I (Negative control) = 12 rats + feed + potable water;

Group II (Positive control) = 12 rats + feed + potable water + BPH induction

Group III (Tamsulosin treated) = 12 rats + feed + potable water;+ BPH induction+ Tamsulosin

Group IV (Fenesteride/Ethanol extract 150 mg/kg): = 12 rats + feed + potable Water +BPH

Group V (Ethanol extract 150 mg/kg) = 12 rats + feed + potable water +BPH induction+ Ethanol extract

water +BPH induction+ Ethanol extract

Group VII (Ethanol extract 600 mg/kg) = 12 rats + feed + potable water +BPH induction+ Ethanol extract

The weights of the rat groups were equalized as nearly as possible. The rats were placed on the same feed (pelletized commercial growers

rat feed from UAC, Nigeria Limited), and they were given feed and water *ad libitum*. All the protocols were approved by the ethical committee of UAES which was in line with the principles of laboratory animal care as designated by the National Institute of Health's Principle (NRC, 1985). The administration lasted for a period of 21 days.

Determination of Body Weight and Visceral Organ Weight

At the end of the treatment period, the rats were weighed before they were anaesthetized with chloroform. Incisions were made at their cervical region and blood samples were collected by cardiac puncture into labeled sterile tubes. The samples were used for further studies. Visceral organs of the liver and kidneys were dissected out and weighed. They were subsequently transferred to 10% formalin-saline solution for preservation till required for further studies.

Body weight change (BOC), Relative body weight (RBW), Organ weight change (OWC), and Relative organ weight (ROW) were calculated as follow;

BOC = Final body weight of rat-Initial body weight of rats

$$\text{RBW} = \frac{\text{Final body weight of rat}}{\text{Initial weight of the rat}} \times \frac{100}{1}$$

Results and Discussion

Table 1 Bioactive profile of *A. muricata* leaf extract.

Bioactive Compound	Qualitative Test	Observation	Inference	Intensity
Alkaloids	Wagner's test	Brick red precipitate	Presence of alkaloids	++
Saponins	Froth Test	No formation of stable froth	Saponins absent	-
Flavonoids	Alkaline reagent test	Yellow colouration	Flavonoids present	++
Terpenoids	Salkowski test	Reddish-brown colouration at the interface observed	Terpenoids present	+
Tannins	Ferric Chloride test	A dirty green precipitate observed.	Tannins Present	++
Phlobatannins	Boiling Acid method	No observation	Phlobatannins absent	-
Phenolics	Liebermann's Test	Deep green coloration observed	Phenolics present	++
Glycosides	Lead Acetate Test	Yellow precipitate observed	Glycosides present	+
Steroids	Liebermann-Burchard Reaction	A change from violet to blue or green coloration observed.	Steroid present	+

Key: - Absent; + Present in moderate concentration; ++ Present in high concentration.

Table 1 shows the present of alkaloids, flavonoids, terpenoids, tannins, phenolics, glycosides, and steroids. Saponins and phlobatannins were not present in the sample. Alkaloids, flavonoids, tannins, and phenolics were found in high concentrations. Terpenoids, glycosides, and steroid were found in low concentrations. These compounds become important when their functions are considered in the biological system. Functionally, the anti-diarrheal, antihypertensive, antifungal, antibrogenic, and anti-inflammatory effects of alkaloids have been reported by Aborisade *et al.* (2017). The potency of some alkaloids against HIV and AIDS has also been reported (McDewvit *et al.*, 1996). Flavonoids in the field of medicine

have been extensively used as antimicrobial, antiviral, antiangiogenic, anticancer, antimalarial and antioxidant (Ullah *et al.*, 2020). Terpenoids have been described as the most abundant compound in natural products and a set of important secondary metabolites in plants with diverse structures. Yang *et al.* (2020) noted that terpenoids possess anti-inflammatory, antitumor, antiviral, antibacterial, and antimalarial effects. The same authors noted that terpenoids promote transdermal absorption, prevent cardiovascular diseases and possess blood sugar lowering potential. Tannins are known for their astringent property and ability to generate physiological response in organisms that consume them. The toxicity of tannins to

some fungal, bacterial, and yeast species has long been established. The presence of tannins in the *A. muricata* leaf sample could be indication of the leaf potential as antifungal, antidiarrheal, antihemorrhoidal and antioxidant agents (Aborisade *et al.* 2017). Sarankar *et al.* (2023) reported that phenolic compounds are a diverse group of compounds found in plants. The same authors noted that many of the phenolic compounds have been utilized for their medicinal properties. Phenolic compounds often possess antioxidant, anti-inflammatory, and other beneficial effects on human health. They are extensively used in medicines (Sarankar *et al.*

2023). Glycosides are among the constituents of medicinal plants and are basically used as cardiogenic, anti-depressant, coloring agent, purgative, diuretic, flavoring agent, antifungal, antidiabetic, anthelmintic, antipyretic, stomachic, and antirheumatic agent (Bhattacharya and Chakraborty 2024). Plant derived steroids have demonstrated diverse bioactivities. They have wide applications in medicine and have shown potentials in the treatment of cancer and inflammatory conditions (Obakan *et al.*, 2023). They are used as natural remedies and as precursors for the synthesis of complex drugs (Obakan *et al.*, 2023).

Table 2 DPPH scavenging activity of the leaf extract.

Concentration ($\mu\text{g/mL}$)	% inhibition	
	Leaf extract	Standard
20	28.97	29.02
40	44.10	41.96
60	55.50	68.03
80	57.70	74.51
100	69.00	83.74

Table 2 shows that % inhibition of the leaf extract ranged from 28.97 % to 69.00% with an IC_{50} value of about 61.34 $\mu\text{g/mL}$ against the standard which ranged 29.02% to 83.74% with an IC_{50} value of 50.64 $\mu\text{g/mL}$. *A. muricata* leaf extract and the standard showed increasing % inhibition as concentration increases. The result confirms a dose-

dependent antioxidant activity. The leaf extract performed comparatively or slightly better than the standard at low concentration (20-40 $\mu\text{g/mL}$) but however reduced at higher concentration (60-80 $\mu\text{g/mL}$). It exhibited strong antioxidant capacity close to the standard at lower doses, suggesting high initial potency. It has been reported that

numerous flavonoids generated as secondary metabolites from different plants have shown to scavenge OH radicals (Baliyan *et al.*, 2022). Inverably, flavonoids present in the leaf extract could be behind the DPPG scavenging potential observed in this study. The IC₅₀ value of *A. muricata* leaf extract (61.34 µg/mL) is higher that the IC₅₀ value of

29.15 µg/mL reported by methanolic extract of *Malva parviflora* by Polile *et al.* (2024), meaning that more of the leaf extract would required to bring about 50% scavenging activities against *M. parviflora*, which would require a much less concentration to achieve same.

Table 3 Nitric oxide scavenging activity of the leaf extract.

Concentration (µg/mL)	% inhibition	
	Leaf extract	Standard
20	23.83	41.59
40	32.70	56.88
60	41.57	59.77
80	58.40	73.22
100	71.51	85.54

Table 3 shows that the % inhibition of the leaf extract ranged between 23.83% to 71.51% , while that of the standard ranged between 41.59% to 85.54%, within the concentration 20 to 100 µg/mL. Both the leaf extract and the standard exhibited a concentration dose dependent increase in % inhibition. However, in all concentrations, the standard exhibited higher activities than the leaf extract. The difference in % inhibition of the leaf extract was wider at low concentrations. The extract reached more than 50% inhibition between 60-80 µg/mL with an IC₅₀ deduced at 70.02 µg/mL while the

standard achieved more than 50% inhibition between 20-40 µg/mL with an IC₅₀ of about 30.00 µg/mL. The standard was more effective in scavenging activity than extract. However, the extract showed good potential, less activity and requires a high concentration to reach 50% inhibition. Nitric oxide (NO) is produced by stresses in the living system (Naqbi *et al.*, 2022). However, it is deleterious in its effects in creating many undesirable effects (Jagatia and Baliga, 2004). The nitric oxide scavenging activity in some medicinal plants has been reported by Jagatia and Baliga (2004) while the antioxidant and

nitric oxide inhibitory activity of some medicinal plants have been demonstrated by Saha *et al.* (2017).

Table 4 Lipid peroxidation scavenging activity.

Concentration ($\mu\text{g/mL}$)	% inhibition	
	Leaf extract	Standard
20	27.83	45.33
40	52.55	56.88
60	63.20	75.55
80	69.40	85.40
100	74.25	95.87

Table of lipid peroxidation scavenging activity (Table 4) shows that % inhibition of the leaf extract ranged from 27.83-74.25% and the standard ranged from 45.33-95.97%, over a concentration range of 20 to 100 $\mu\text{g/mL}$. The inhibition of the leaf extract crossed 50% between 20

$\mu\text{g/mL}$ and 40 $\mu\text{g/mL}$ with IC_{50} of 37.94 $\mu\text{g/mL}$ for the extract and 28.08 $\mu\text{g/mL}$ for standard. The extract showed a moderate lipid peroxidation activity when compared with the strong peroxidation activity of the standard, and could be a potent source for antioxidant.

Table 5 Reducing power of the leaf extract.

Concentration ($\mu\text{g/mL}$)	Absorbance	
	Leaf extract	Standard
20	0.116	0.146
40	0.236	0.341
60	0.304	0.409
80	0.423	0.456
100	0.548	0.632

The reducing power of the leaf extract over a concentration range of 20-100 $\mu\text{g/mL}$ shows absorbance range of 0.116- 0.548 for the leaf extract and absorbance range of 0.146-0.632 for the standard (Table 5). The absorbance values revealed that *A. muricate* leaf extract

increase with concentration and an IC_{50} value of 51.2 $\mu\text{g/mL}$. The same dose dependent pattern was exhibited by the standard with an IC_{50} value below 20 $\mu\text{g/mL}$. The standard however exhibited higher absorbance values for all the concentrations, suggesting a

stronger electron donating potential. Altogether, the IC₅₀ of the leaf extract indicates moderate antioxidant potency. Naqbi *et al.* (2022) noted that the antioxidant activity of a given extract or molecule can be evaluated based on reducing capacity. According to Ou *et al.* (2002), the general property of a compound inducing the reducing power is associated with the breaking of free radical chain with the provision of a donation of a hydrogen atom. The absorbance of *A. muricata* leaf extract clearly increased due to

the formation of Fe²⁺ complex with higher contents. This could be attributed to the tannin content of the leaf extract. Okuda (2005) noted that tannins possess ferric (Fe³⁺) reducing ability. The observation of the present study is in line with the work of Naqbi *et al.* (2022) on the reducing power of *Calligonum crinitum* Boiss. The IC₅₀ of the leaf extract in the present study is lower than the IC₅₀ of methanolic extract of *Malva parviflora* leaves collected in Labanon (Abdel-Ghani *et al.*, 2013).

Table 6 Body weight of rats

Body weight (g)	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
Initial body weight	163.10	164.57	164.01	164.10	164.00	164.00	164.00
Final body weight	264.80	196.00	224.76	218.86	210.75	208.10	217.27
Weight change	100.90	31.43	60.75	54.76	46.75	44.10	53.27
% weight Change	61.86±0.13 ^e	19.10±0.03 ^a	37.04±0.01 ^d	33.37±0.12 ^c	28.51±0.14 ^b	26.89±0.24 ^b	32.48±1.05 ^c
RBW (%)	162.35±1.01 ^f	119.10±1.05 ^a	137.04±1.03 ^e	133.37±1.15 ^d	128.50±1.19 ^e	126.89±1.57 ^b	132.48±1.20 ^d

Results are presented for initial and final body weights, and weight changes; while results of % weight change and RBW are presented as mean and standard deviation of triplicate determination. Values with different letters of alphabet are statistical significant at p<0.05.

Table 6 shows that initial body weight of the rats ranged from 163.01 g in groups I to 164.67 g in group II. The final body weight of the rats ranged from 196.00 g in group II to 264.80 g in group I. Weight change ranged

from 31.45 g in group II to 100 g in group I. % weight change ranged from 19.10% in group II to 61.86% in group I. RBW for the rats ranged from 119.10 in group I to 162.35 in group II. It has been noted that body weight plays an important role in toxicological studies, especially in determining how substances affect living organisms. The weight change of the rats ranged from 31.43g in group II to 100.90 g in group I. The % weight change ranged from 19.10% in group II to 61.86% in group I. The significant

($p < 0.05$) reduction in body weight of the rats induced with BPH (groups II to groups VII) against group I (negative control) signaled the toxic effect of the induction on the body weight of the rats. However, the significant ($p < 0.05$) increase in body weight observed in groups III to VII rats when compared to group I rats (positive control) showed a rapid improvement in body weight as a result of the treatment given to the rats. The extract could be responsible for the significant ($p < 0.05$) increase in body weight of the rats as observed in groups V to VII against the positive control rats (Group II). Invariably, the extract could possess health improvement potential.

Conclusion

The bioactive profile observed in *A. muricata* in this study include of alkaloids, flavonoids, terpenoids, tannins, phenolics, glycosides and steroids. Alkaloids, flavonoids, tannins, and phenolics were found in high concentrations. Terpenoids, glycosides, and steroid were found in low concentrations. Also, the leaf extract exhibited moderate scavenging potential against different standard antioxidant agents when tested with different standard methods. Experimental animals induced with BPH and placed on the leaf extract, massively recovered in body weight

against those treated with BPH pharmaceutical drugs, and as well showed improvements when compared with positive control. These observations could be attributed to the bioactive profile identified in the leaf extract. This study has revealed the bioactive profile, antioxidant potential, and functional attributes of *A. muricata* leaf extract on weight in rats induced with BPH,

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