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Anti-diabetic and Toxicological Evaluation of Acetone Fruit-peel extract of *Annona muricata* in Streptozotocin-induced Wistar rats

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ABSTRACT

Medicinal plants have been used since time immemorial for the management of diseases due to their potency, low cost and availability. However, they can be toxic due to their chemical make-up, hence the need for toxicity evaluation. Fruit peels of *Annona muricata* were collected, authenticated, processed and extracted using maceration technique. Extract was subjected to phytochemical, and antioxidant analyses using Harborne's method, and DPPH assay respectively. Antidiabetic activity and toxicity on streptozotocin induced diabetic and healthy Wistar rats were analysed by fasting blood glucose levels, acute and sub-acute toxicity tests respectively. Data were analysed and statistical significance was considered when *p-value* was < 0.05. Bioactive compounds detected included: phenol, tannin, flavonoids, terpenoids, saponin, cardiac glycosides, alkaloids and coumarins. The extracts scavenged free radicals revealed in a dose-dependent manner: 1C50 values range: 34.97 µg/ml -144.86 µg/ml; AAI value range: 5.5-23.5. Acetone had the highest antioxidant capacity (AAI value: 23.5). Mean weight of the diabetic rats treated with normal water declined with a significance difference to those treated with the extracts ($p<0.05$). Extracts of *A. muricata* at 1000 mg/kg reduced blood glucose level of diabetic induced rats from 207.0 ± 1.73^{a} to 87.0±2.65 mg/dl (n=5), compared with the standard antidiabetic drug 84.7 ± 1.15^{a} ($p>0.05$) respectively. Death was not observed in both acute and sub-acute toxicity studies, the liver exhibited no anatomical changes, the kidney showed active interstitial congestion and inconspicuous tubular injury. *Annona muricata* acetone extract exhibited antidiabetic potential with no acute toxicity, but the kidney showed active interstitial congestion and inconspicuous tubular injury.

Keywords: *Annona muricata*, Antidiabetic, Bioactive compounds, Medicinal plants, Toxicity

INTRODUCTION

Diabetes has conventionally been considered a 'rich disease' among elderly people especially in the developed countries. However, it is now disturbing all levels of society and is rapidly becoming a growing problem in poor communities (Safiri *et al.*, 2022). Diabetes is one of the

major global health crises with a projection of diagnosis of 783 million people worldwide by the year 2045 (Magliano and Bokyo, 2021).

Belonging to the Annonaceae family, *Annona muricata Linn*. has recently attracted the attention of scientists for its medicinal value. It has been utilized as a medicinal remedy since ancient times to treat and improve various diseases, for example, diabetes mellitus, hypertension, cancer, and bacterial infections (Mutakin *et al*., 2022., Zubaidi *et al.*, 2023). The Annonaceae family contains different types of bioactive chemical properties, such as alkaloids, acetogenins, flavonoids, terpenes, and essential oil, meaning the plants in this family are potential therapeutic agents (Mutakin *et al*., 2022). Many studies show that soursop leaves have a range of biological activities such as wound healing, antimicrobial, induces apoptosis, antioxidant, cytotoxic activity on cancer cell without affecting a normal cells, anti-diabetic and free radical scavenging activity (Moghadamtousi *et al*., 2015). The biological activity of *A. muricata* is thought to be mediated by phytochemicals including acetogenins (annomuricins and annonacin), alkaloids (coreximine and reticuline), flavonoids (quercetin), and vitamins (Mutakin *et al*., 2022).

Fruit peels are rich reservoirs of bioactive compounds with therapeutic potentials for human health (Koushik *et al*., 2025). These bioactive compounds have been reported to exhibit strong antioxidant properties and other diverse biological activities like antimicrobial, antidiabetic and anticancer (Koushik *et al*., 2025). According to Shu *et al*. (2020), a number of chemicals that are part of the phenolic and flavonoid groups, such as nobiletin, tangerine, and coumaric acid, have been isolated and measured from orange peel utilizing their capacity to scavenge radicals. The bioactive compounds that are extracted from fruit wastes have hypoglycaemic, antimicrobial and strong anti-inflammatory properties (Sha *et al*., 2023).

Globally, 80% of the populace use plant-derived products for treating or preventing diseases with the general perception that they are safe and devoid of adverse effects. However, approximately 150000 plants have been reported to contain toxic substances harmful to the body when used for a prolonged time at a particular dose (Mugale *et al*., 2024). In recent years, the majority of conducted studies on the plant have only focused on major parts of the plants, and there is scarcity of information with regards to biological activities like the antidiabetic activity of the waste parts of the plants, hence the nutritional and functional characteristics of soursop peels warrant further investigation for the purpose of extracting bioactive compounds that may be utilized in reducing the blood glucose levels in diabetic patients and also assessing their therapeutic usages by determining their toxicity profiles. This study therefore evaluates the "antioxidant, antidiabetic and sub-acute toxicity profiles of acetone and methanol fruit peel extracts of *A. muricata* in STZ-induced Wistar rats.

MATERIAL AND METHODS

Collection, identification and extraction of plant material

The fruit of *Annona muricata* was collected from Ogun state, Nigeria, and properly identified by Mr Esimekhuai, D. P. O. at Pharmacognosy Department, University of Ibadan, Oyo State where sample was deposited and assigned voucher number (UIH-23541) on the 3rd of March, 2025.

The fruit was washed with distilled water and the peel extracted and chopped with knife into smaller pieces. The peels were allowed to air dry for a month under the shade in a dry room with good ventilation and low humidity at room temperature to prevent microbial contamination. The dried fruit peel was grounded using a wooden mortar and pestle into coarse powder and later finely grinded with the aid of an electric blender and stored at room temperature in an air-tight container, protected from sunlight until required for further use.

Fifty gram (50g) each of the powdered fruit peel of *Annona muricata* was macerated for three days at room temperature in 500 mL acetone and 500 mL methanol (1:10 w/v) for 72 h at room temperature with repeated stirring. The extract obtained was filtered with Whatman No. 1 filter paper and concentrated in vacuum by rotary evaporation (Buchi, R-215, Germany) at reduced pressure and 30°C. The concentrated extracts were dried in a water bath (60°C) until the organic solvent evaporates. and kept in the freezer (–25°C) until further analysis. Percentage yield was then calculated as:

$$\text{Percentage Yield} = \frac{\text{Weight of Extract}}{\text{Weight of Crude Drug}} \times 100\ \%$$

Preliminary phytochemical analysis of the extracts.

The methanol extract (MAM) and acetone extract (AAM) was subjected to various qualitative phytochemical tests to determine the presence of various phyto-constituents using the method of Harborne as described by Onabanjo *et al*. (2020).

Alkaloid

The alkaloid test was performed by adding 1 millilitre of the crude filtrate solution and a few drops of Wagner's reagent

to a test tube containing 2 millilitres of chloroform, and the results were recorded.

Tannin

To investigate the presence of tannins, to a portion of crude filtrate solution in distilled water, about 4 drops of 20% ferric chloride solution were carefully added and observed for colour change.

Terpenoids

By mixing 1 millilitre of crude extract solution with 2 millilitres of chloroform and then adding 1 millilitre of acetic anhydride, one millilitre of concentrated sulfuric acid was added to the solution and the result recorded.

Flavonoids

Few drops of diluted sodium hydroxide solution were added to 0.5 ml extract. A deep yellow colour mixture which became colourless upon the addition of few drops of diluted H_2SO_4 acid indicates the presence of flavonoids.

Steroids

About 0.5 ml of acetic anhydride and few drops of concentrated H_2SO_4 were added to 1 ml of extract filtrate, and it was later observed.

Anthraquinone

To 10 ml of chloroform, 1 ml of the crude filtrate solution was added to the extract and observed.

Phenol

Five (5 ml) of the extract was pipetted into a 30 ml test tube, then, 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated alcohol were also added and left to react for few min. it was later observed.

Saponin

Ten (10 ml) of water was added to 1ml of the extract and vigorously shaken for 5-10 minutes, it was observed for the appearance of froth.

Cardiac glycoside

One (1 ml) of glacial acetic acid and 5 % ferric chloride was added. To 2 ml of plant extract followed by the addition of 3 drops of concentrated sulphuric acid.

Coumarins

Drops of $FeCl_3$ was added to the extract, a dark green colour turning yellow upon addition of HNO_3 suggests he presence of coumarins.

Antioxidant analysis using 1, 1-diphenyl-2-picryl-hydrazyl (DPPH)

The method of Ganesan *et al*. (2024) was used to assess the antioxidant potentials of methanol (MAM) and acetone (AAM) extracts of fruit peels of *Annona muricata*. An 0.1 mM solution of 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) in ethanol was prepared, and 1 ml of the solution was added to 1 ml of extract in water at different concentrations (50-800µg/ml). The mixture was shaken vigorously and left to stand at room temperature for 30 minutes. The absorbance was then measured at 517 nm using a UV-visible spectrophotometer; the lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Gallic acid was used as a standard reference, and the absorbance of a blank without the antioxidant was also measured, and the values were subtracted from the optical density of the samples. All the determinations were performed in triplicates and expressed as Mean ± SD. The percent DPPH scavenging effect was calculated using the following equation

$$\text{DPPH Scavenging effect (\%)} = [(A_0-A_1)/A_0] \times 100$$

Where A_0 was the absorbance of the blank and A_1 was the absorbance in the presence of the standard sample or extract. The IC_{50} value which is the concentration of the extracts that caused 50% inhibition of DPPH radical formation were determined using a dose-response logarithmic fitting curve. The Antioxidant Activity Index (AAI) was calculated as shown below.

AAI= The final concentration of DPPH/ IC50

According to the criteria of Sherer and Godoy (2009), plant extracts with AAI less than 0.5 shows poor antioxidant activity, 0.5-1.0 (moderate), 1-2 (strong) and AAI above 2 shows very strong activity.

Antidiabetic analysis.

Healthy male Wistar rats weighing averagely 180g (6- 8 weeks old) were acquired from the Faculty of Pharmacy Animal house (FPAH), Olabisi Onabanjo University Sagamu Campus, Sagamu, Ogun State. The rats were kept under standard laboratory conditions with free access to water and standardized pelletized feed. They were allowed to acclimatize for two weeks before the commencement of the study. The study was approved by Health Research Ethics Committee (HREC), Olabisi Onabanjo University Teaching Hospital, Sagamu, Ogun State, Nigeria (OOUTH/HREC/674/2023AP). Animals were treated ethically according to the guidelines provided by National Institute of Health (NIH, United State).

Diabetes was induced with single dose of 55 mg/kg body weight streptozotocin (Sigma-Aldrich, Saint. Louis) intraperitoneally in normal rats fasted for 16 hours. D-glucose (3g/kg) was administered after one hour of induction to prevent hypoglycaemic shock and those having blood glucose levels greater than or equal to 200mg/dL were considered diabetic and selected for the experiment. after been fasted overnight was regarded as diabetic (Awote *et al.*, 2024). A total of 25 male Wistar rats were divided into five group each of five animals as follows
R1: Diabetic control (Received only 10mL/kg of water orally)
R2: Positive control (Received 5mg/kg of glibenclamide orally)
R3, R4 and R5 received 500 mg/kg, 1000 mg/kg and 1500 mg/kg of acetone extract of *Annona muricata* fruit peel respectively. The acetone extract was chosen for the antidiabetic study due to its higher antioxidant activity as reported from the present study. High antioxidant activity of a plant is linked to their antidiabetic potential (Dilworth *et al.*, 2024).

The rats were treated orally with the treatment options above for 9 days and the blood glucose levels were taken after 72, 120,168 and 216 hours with the aid of glucometer (Accu-check Test meter) while the weight were taken with the aid of weighing balance after 120 and 216 hours.

Toxicity analysis

Acute-toxicity. Acute toxicity of acetone extract of *Annona muricata* fruit peel was evaluated in Wistar rats using Lorke's model as reported by Opara *et al.* (2024). Healthy Male Wister rats weighing averagely 200g (6- 8 weeks old) that has been acclimatized for two weeks were orally fed with different concentration per body weight. The model consists of two phases comprising of 18 animals. The first phase which is of lower concentration was between 10 mg/kg – 1200 mg/kg/body weight doses while the second phase is made up of the larger doses from 1600 – 5000 mg/kg/body weight doses. The experiment was carried out for forty hours starting with the lower doses and after confirming that the treated animals survived, then the second phase was carried out. At each occasion, the animals were been observed for any manifestation of toxicity for at least 24 hours.

Sub- acute toxicity

The sub-acute oral toxicity was conducted in accordance with the guidelines of OECD (2008). Rats were randomly divided into four groups of three animals each. Group one served as the normal control and did not receive the extracts, while group 2-4 received the acetone extract at 500 mg/kg, 1000 mg/kg and 1500 mg/kg respectively for 28 days. The rats fed with the highest dose (1500 mg/kg) were sacrificed by cervical dislocation technique, carefully opened up and the organs harvested with the aid of a forceps and a pair of scissors. The organs were kept to fix in 10% formaldehyde solution. All harvested organs were preserved in 10% normal formaldehyde for 24 hours and transferred to the Histopathology Laboratory, Olabisi Onabanjo University, Sagamu Campus, Sagamu, Ogun State for histological analysis. Organs including kidney and liver were sectioned at 5 microns and stained using Haematoxylin and Eosin (H and E) techniques for the general tissue-organ architecture, sections were viewed under the light microscope at x400 magnification and photomicrographs were obtained using a scope exe camera attached to a computer.

Data analyses: Data obtained were analysed using SPSS software version 20.0. Descriptive statistics such as frequency distribution, percentages, and means and corresponding standard deviations; and statistical differences between control and treatment groups were evaluated using one way ANOVA followed by post Hoc test (Dunnett's). The statistical significance of all results was considered when the p value was < 0.05.

RESULTS

Preliminary phytochemical analysis of extracts of *Annona muricata* fruit peel

Table 1 presented the preliminary qualitative bioactive contents of the extracts of *A. muricata*. Phenol, saponin, flavonoids and terpenoids were detected in all the extracts with none showing positive results for steroids and anthraquinone. Cardiac glycosides and coumarins and alkaloid were present in AAM but absent from MAM.

Table 1. Phytochemicals present in extracts of *Annona muricata* fruit peel

Phytochemical test	AAM	MAM
Phenol	+	+
Tannin	-	+
Saponin	+	+
Anthraquinone	-	-
Flavonoids	+	+
Terpenoids	+	+
Cardiac glycosides	+	-
Coumarins	+	-
Steroids	-	-
Alkaloid	+	-

Keys: MAM= Methanol extract of *A. muricata*, AAM= Acetone extract of *A. muricata*. += presence of phytochemicals, - = absence of phytochemicals

Table 2 depicts the absorbance of the extracts and the standard control at 517 nm using DPPH assay. the absorbance at different concentration was recorded and the mean ± standard deviation of the tree replicate was calculated. At 800µg/ml, the extracts of gallic had absorbance of 0.002±0.005, AAM (0.144±0.05), MAM (0.202±0.005). The absorbance of the extracts and gallic acid was concentration dependent, the lower the concentration, the higher the absorbance.

Figure 1 presented the DPPH radical scavenging activity of AAM, MAM and gallic acid. The scavenging activity of the extracts at 800 µg/ml was comparable to that of the standard antioxidant (gallic acid). Figure 2 and 3 depicted the graphical representation of the 1C50 using a logarithmic fitting curve.

Table 3 presented the IC50 and the AAI of the extracts with MAM having the highest IC50 at 144.864 µg/ml. All the extracts had AAI values above 2, which suggest them as very strong antioxidant agents.

Table 2: Absorbance of DPPH assay by fruit peel extract of *Annona muricata* at 517 nm

Extracts	Concentration (µg/ml) / Absorbance at 517nm				
	50 µg/ml	100 µg/ml	200 µg/ml	400 µg/ml	800 µg/ml
Gallic acid	0.107±0.00^{c}	0.102±0.001^{c}	0.072±0.01^{b}	0.045±0.03^{b}	0.002±0.00^{a}
AAM	1.228±0.00^{c}	0.194±0.00^{b}	0.175±0.00^{ab}	0.159±0.00^{ab}	0.144±0.01^{a}
MAM	1.602±0.05^{e}	1.133±0.31^{d}	0.572±0.01^{c}	0.326±0.01^{b}	0.202±0.01^{a}

Values are triplicates expressed in mean ± standard deviation and those bearing the same superscripts are not significantly different (P > 0.05).

Keys: MAM= Methanol extract of *A. muricata*, AAM= Acetone extract of *A. muricata*.

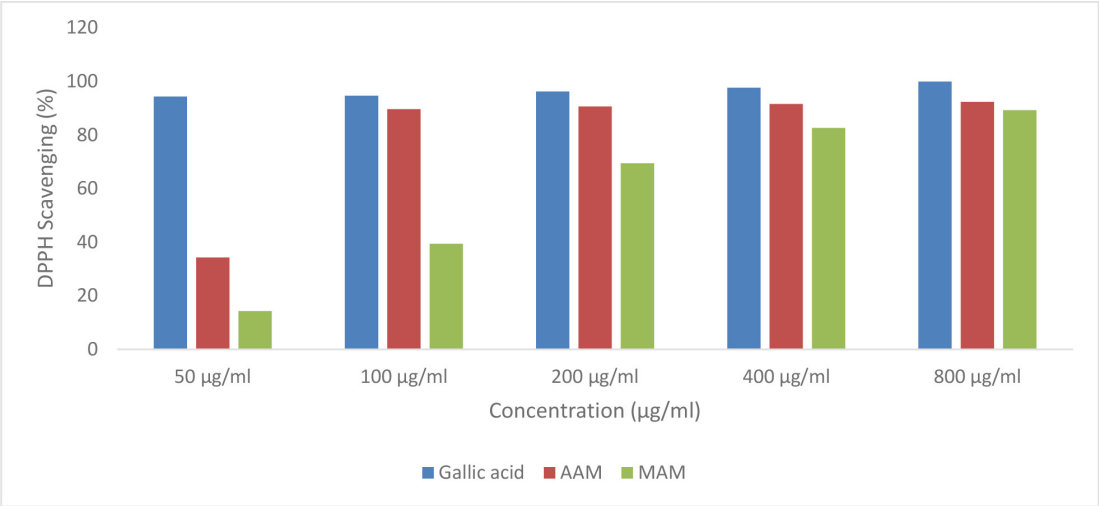

Figure 1: DPPH radical scavenging activity of extract of *Annona muricata* fruit peel

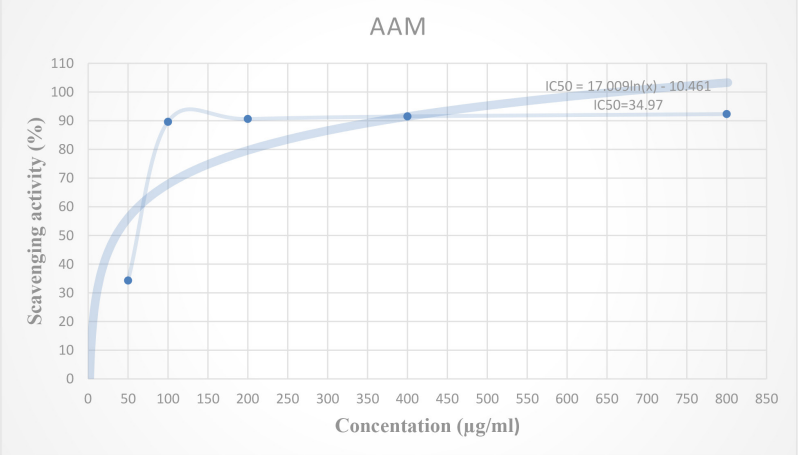

Figure 2: Inhibitory concentration (IC50) of acetone extract of *Annona muricata* fruit peel
*(IC50) =plot % inhibition vs log concentration

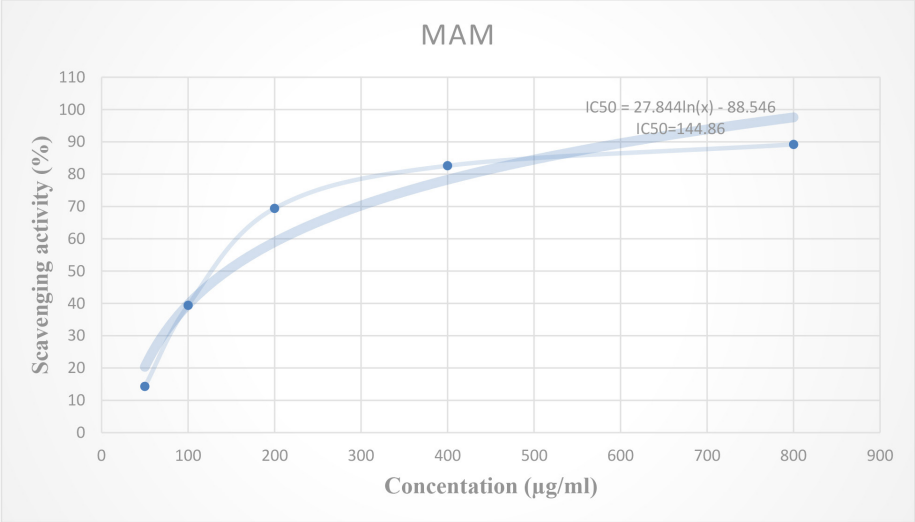

Figure 3: Inhibitory concentration (IC50) of acetone extract of *Annona muricata* fruit peel
*(IC50) =plot % inhibition vs log concentration

Table 3: Antioxidant activity index (AAI) of fruit peel extracts of *Annona muricata* using DPPH assay

Extract	IC50 (µg/ml)	AAI*(800 µg/ml/IC50)	Category of antioxidant
AAM	34.972	23.5	Very strong
MAM	144.864	5.5	Very strong

Keys: MAM= Methanol extract of *A. muricata,* AAM= Acetone extract of *A. muricata*.

Antidiabetic activity of fruit peel extracts of *Annona muricata*.

The abilities of the extracts to reduce blood glucose levels in rats that have been induced with diabetic causing drugs (Streptozotocin) was tested. The weight of the rats after the induction of diabetes was measured at two different time intervals (120 hours and 216 hours) as shown on Table 4. The mean weight of the diabetic rats treated with normal water declined after the treatment hours with no significant differences in mean weight over the period of treatment but significant difference in mean weight of those treated with glibenclamide and the extracts at ($p< 0.05$). There was a slight increase in the mean weight of rats treated with glibenclamide and acetone extract of *Annona muricata* fruit peel after the 216 hours treatment with no significance difference among the mean weight of rats treated with glibenclamide to those treated With AAM at ($p > 0.05$).

The hypoglycaemic effect of fruit peel of *Annona muricata* is as shown on Table 5. The study revealed that the crude extracts of AAM exhibited hypoglycaemic activity at 500mg/kg, 1000mg/kg and 1500mg/kg against streptozotocin induced Wister rats at 72, 120, 168 and 218 hours. Their results differed significantly to the negative control (water). At 72 hours, there was no significant differences in the blood glucose level at all concentrations used at ($p > 0.05$). At 120 and 168 hours, there was significant difference between the positive control (glibenclamide) and the other treatment at ($p< 0.05$). At 216 hours, there was no significant difference between the positive control and AAM extracts at 500 and 1000mg/kg. The hypoglycaemic effect of fruit peel of *Annona muricata* was comparable to the positive control at 1000 mg/kg.

Table 4: Weight (g) of the experimental rats after induction of hyperglycaemia and treatment with Acetone extract (AAM)

Treatment	Mean weight (g)	
	After 120 hrs	After 216 hrs
Glibenclamide	202.0±2.00^{c}	208.8±0.83^{c}
Water	167.4±1.14^{a}	151.4±0.89^{a}
500 mg/kg	201.0±1.22^{c}	210.0±1.00^{c}
1000 mg/kg	202.0±2.00^{c}	207.2±0.83^{b}
1500 mg/kg	198.0±1.41^{b}	208.8±0.83^{c}

Values are expressed as mean standard deviation (n=5) and those bearing the same superscript are not significantly different at ($p > 0.05$)

Table 5: Fasting blood glucose (mg/dL) in streptozotocin induced diabetic rats treated with Acetone extract (AAM)

Treatment	Zero hour	72 hours	120 hours	168 hours	216 hours
Glibenclamide	207.0±1.00[a]	140.0±1.00[a]	108.0±1.73[a]	109.7±0.58[a]	84.7±1.15[a]
Water	208.0±1.00[a]	243.0±1.00[e]	267.3±2.08[e]	279.7±1.53[c]	317.7±1.15[c]
500 mg/kg	207.3±1.53[a]	159.6±1.53[d]	127.3±1.53[d]	122.7±2.08[b]	103.7±1.15[b]
1000 mg/kg	207.0±1.73[a]	156.3±1.53[c]	121.0±1.00[b]	121.7±1.15[b]	87.0±2.65[a]
1500 mg/kg	207.2±1.49[a]	153.7±1.00[b]	124.3±0.58[c]	124.0±1.73[b]	103.6±2.52[b]

Columns represent values that are expressed as mean ± standard deviation (n=5) and those bearing the same superscript are not significantly different at *(p > 0.05)*

Toxicity evaluation of fruit peel extracts of *Annona muricata* on Wistar rats

Acute toxicity profile of acetone extract of *A. muricata* is presented on Table 6. The acute toxicity test was carried out in two phases at concentrations first, then higher concentration. There was no death recorded at both phases even at concentration as high as 5000 mg/kg on Wistar rats used in the study with LD50 >5000 mg/kg.

The subacute effect of the extract on the weight of the rat liver and kidney after repeated exposure for 28 days is as shown on Table 7 while Figure 4 and 5 depict the liver histopathology of the rats at a dose of 1500 mg/kg. There was no significance difference in the liver weight of control and AAM. For the weight of the kidneys, there was significance difference among all the control and the extracts. The liver of rats fed with AAM revealed a regular tubular nucleoli and healthy vesicular nuclei which is similar to the liver of the rat fed with normal water.

Figure 6 and 7 showed the kidney histopathology of rats fed with water and AAM respectively. The kidney of the rats fed with AAM showed mild active interstitial congestion and inconspicuous tubular injury after exposure to the extracts for 28 days.

Table 6: Acute toxicity profile of acetone extract of *Annona muricata* fruit peel

Phase one group	No of animals	Dosage of extracts (mg/kg)	No of death	Behavioural changes
1	3	10	0	None
2	3	100	0	None
3	3	500	0	None
4	3	1000	0	None
5	3	1200	0	None
Phase two group				
1	1	1600	0	None
2	1	2700	0	None
3	1	5000	0	None
Total	18		0	None

Note: LD50>5000 mg/kg

Table 7: Mean weight of liver and kidney after 28 days sub-acute evaluation at 1500mg/kg dose Acetone extract (AAM).

Extract (1500 mg/kg)	Mean weight of kidney (g)	Mean weight of liver (g)
AAM	0.867±0.688[a]	5.933±0.031[b]
Water	1.28±0.020[c]	6.050±0.700[b]

Values are expressed as mean standard deviation (n=3) and those bearing the same superscript are not significantly different *(p > 0.05)*.

Keys: AAM= Acetone extract of *A. muricata*.

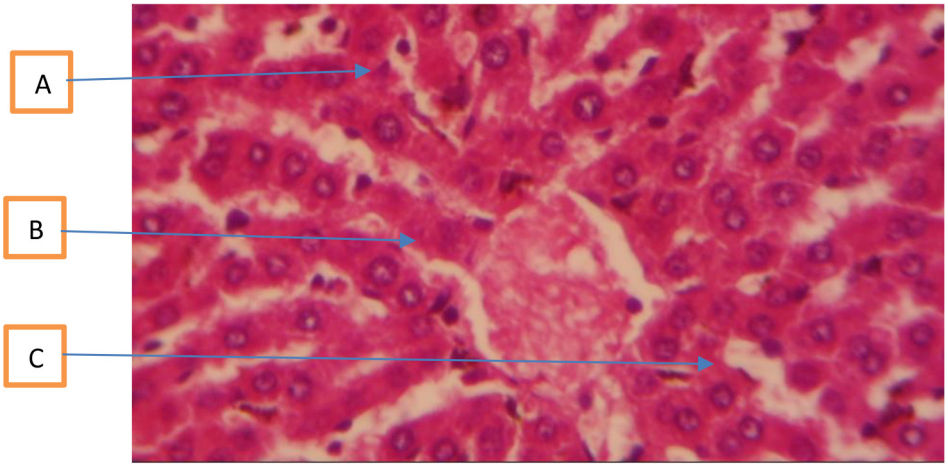

Figure 4: Photomicrograph showing the liver for the normal control fed with water (H&E x 400)

Keys: A: hepatocytes, B: sinusoids and C: central vein

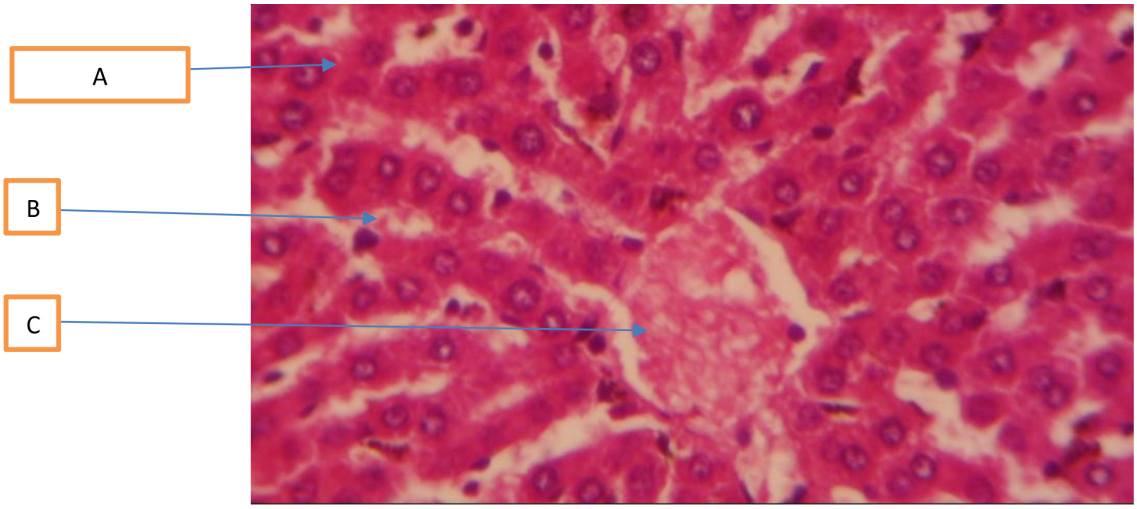

Figure 5: Photomicrograph showing the liver of the rat fed with 1500mg/kg of acetone extract of *Annona muricata* fruit peel (H&E x 400)

Keys: hepatocytes cell B: sinusoids and C: central vein

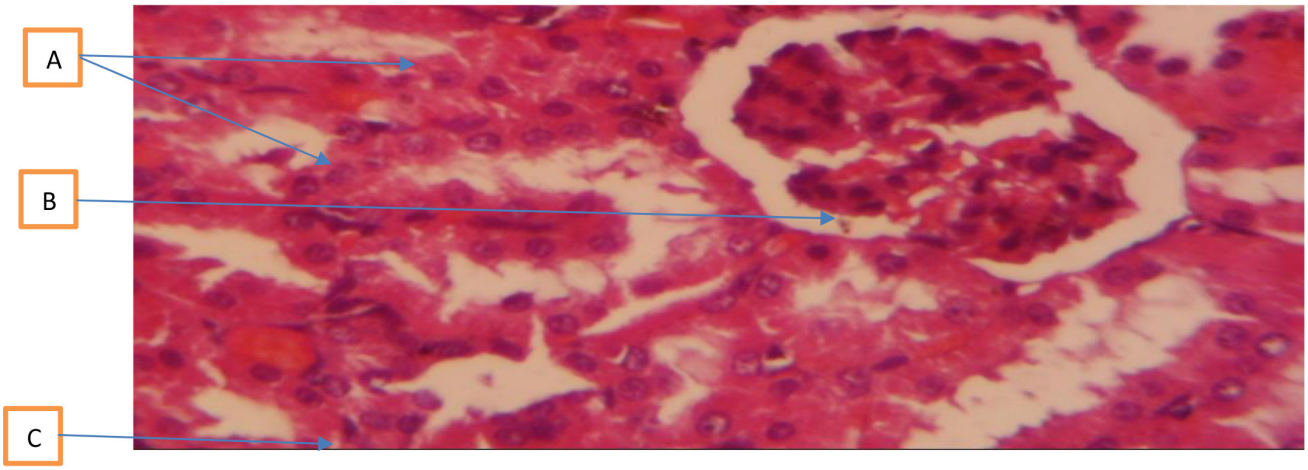

Figure 6: Photomicrograph showing the kidney for the normal control fed with water (H&E x 400)

Keys: A: tubules, B: glomerulus and C: interstitial space

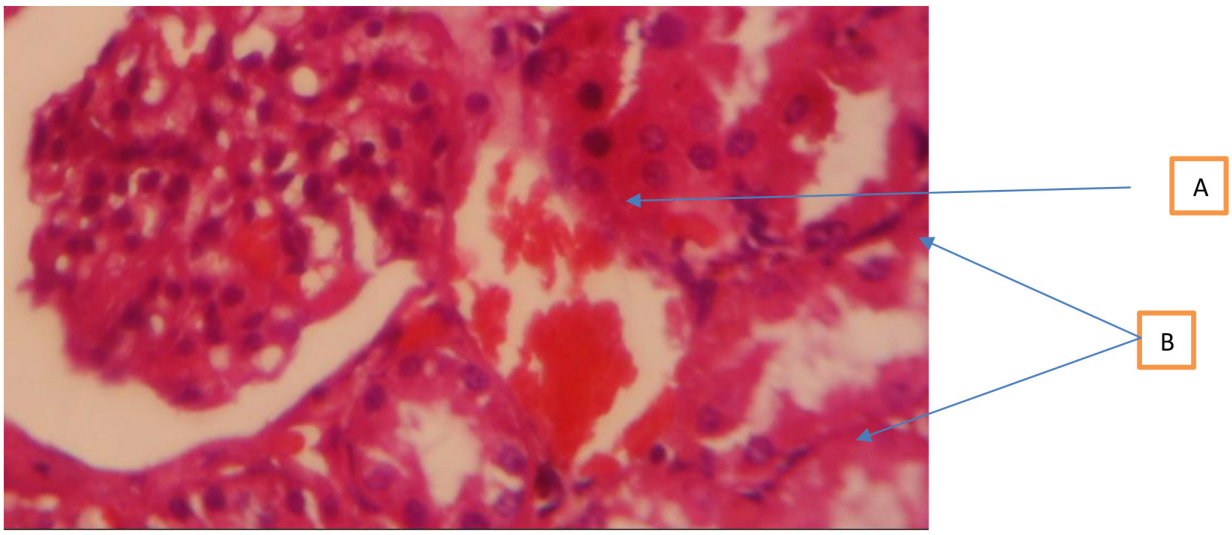

Figure 7: Photomicrograph showing the kidney of the rat fed with 1500mg/kg of acetone extract of *Annona muricata* (H&E x 400)

Keys: A: active interstitial congestion and B: inconspicuous tubular injury

DISCUSSION

The qualitative phytochemical analysis of *A. muricata* is in accordance with the report of Prasad *et al.* (2021) that investigated the antibacterial activity and the phytochemical analysis of ethanolic leaf extract of *A. muricata*. The study revealed the presence of alkaloids, flavonoids, protein, carbohydrate, glycosides, tannins, terpenoids and anthraquinones. The presence of flavonoids, phenol, tannin and alkaloids makes the plants useful as antidiabetic, antibacterial and antioxidants as it helps in preventing oxidative stress and hastening of wound healing (Ajiboye *et al.*, 2018). Saponin lowers cholesterol and blood glucose levels (Salim *et al.*, 2023).

Antioxidants agents are free radical scavenging molecules that prevents or delay cell damage by neutralizing harmful free radicals that could occur as a result of cell ageing, diseases and lifestyles (Taha *et al.*, 2021; Kanwal *et al.*, 2024). The extracts were able to scavenge free radicals from the DPPH assay in a dose-dependent manner, that is comparable to the reference antioxidant (gallic acid). The high scavenging activity of *A. muricata* at 92.3% is in accordance with the reports of other studies that reported high percentage scavenging inhibition (Vieira *et al.*, 2022; Abdukareem *et al.*, 2024). Antioxidant activity index of a plant extract can be determined using DPPH where antioxidant activity could be classified as poor, moderate, strong and very strong with AAI value of <0.05, 0.5≤1, 1≤ and≤2, and >2 respectively (Schreder and Godoy, 2009). According to the criteria of Sherer and Godoy (2009), the AAI of the plant extracts had a very strong antioxidant property with AAI value of 5.5 for MAM and 23.5 for AAM which is in similar with the AAI value of fruit peel extract of *Artocarpus heterophyllus* from a study of Insanu *et al.* (2022), that recorded AAI value of 36.8± 2.8.

One of the major features of diabetes mellitus is the high concentration of glucose caused by insulin deficiency and persistence of hyperglycaemia may affect major organ like brain, liver and kidney in the body (Navarro-Gonzalez, 2013; Ajiboye *et al.*, 2020). The immune suppression caused by diabetic infection promotes the emergence of novel strains of MDR pathogens (Darwitz *et al.*, 2024). Extracts of *Annona muricata* fruit peel used in the study were able to lower the blood glucose level of streptozotocin induced diabetic rats which compared favourably with the standard antidiabetic drugs used in the study. The hypoglycaemic effect of the extracts could be attributed to various secondary metabolites present in the extract, though further studies is required to substantiate the hypoglycaemic effects.. Phytochemicals from medicinal plants have been reported as therapeutic agents against diabetes (Monika and Dhingra, 2023). The extracts of AAM increased the body weight of the treated diabetic rats at a value comparable to the weight increase caused by the standard drug (Glibenclamide) at all concentrations. There was no significance difference in the weight gain at 500mg/kg and 1500mg/kg but a significant difference at 1000mg/kg. This report is in accordance with the report of the study of Akinlolu *et al.* (2023), that reported a reduction in body weight of streptozotocin induced diabetic rats treated with ethanolic extracts of *Annona muricata* ripe fruit pulp. The diabetic rats treated with normal water showed reduction in body weight that was

significantly different when compared with other rats treated with the extracts and positive control. The study of Zhang *et al.* (2022) reported the antidiabetic potential of fruit peel of orange in diabetes management. An in-vivo study on the antidiabetic effect of fruit peel of orange at 300 and 600mg/kg on diabetic rats recorded a significant reduction in the glucose levels which is in line with the present study. Ajiboye *et al.* (2018) reported the anti-hyperglycaemic effect of *A. heterophyllus* stem bark on diabetic rats. Manurung *et al.* (2021) also reported the ability of seed extract of *A. heterophyllus* to reduce blood sugar levels in female Wistar rats with gestational diabetes mellitus induced by streptozotocin. The anti-diabetic activity of *A. muricata* has also been reported to lower the blood glucose levels (Opara *et al.*, 2021). Anti-diabetic effect of the acetone extract was not dose dependent as the glucose level of the rats at concentration above 1000 mg/kg increased which could be attributed to the presence of carbohydrates in the extract, insulin resistance or toxicity of the extracts.

In the present study, the acute toxicity of acetone extract of fruit peel of *Annona muricata* on the experimental rats showed no mortality even at a higher concentration of 5000 mg/kg. This is in line with the report of Arthur *et al.* (2011) who recorded no mortality at 5000 mg/kg but in contrast with the study of Opara *et al.* (2024) that reported the acute toxicity of leaf ethanol extract of *A. muricata* at concentration above 2900 mg/kg. The result of the study is also in agreement with other studies (Olowofolahan *et al.*, 2021; Al-Medhtiy *et al.*, 2022; Opara *et al.*, 2024). Extracts with LD50 greater than 3000-5000 mg/kg could be considered of low toxicity and safe for oral administration (Opara *et al.*, 2024). Sub-acute evaluation is a means of determining the safety profile of plant extracts following repeated exposure for 28 days on rodents (Christophe *et al.*, 2017), and also shows the cumulative effects on major organs (Loha *et al.*, 2019). The parameters used in checking the long-term toxicological effects of extracts of *A. muricata* fruit peel are the organ weight and histopathological changes in the organs of rats. There was no death reported after the 28days treatment which buttress the no death recorded in the acute study but there was significant reduction in the weight of liver and kidney of rats fed with 1500 mg/kg of both extracts which could be attributed to some toxic agents from the plant extracts. The toxic effect of plant extracts usually affects target key organs like liver, kidney, heart and spleen (Anywar *et al.*, 2021). The presence of tannin and saponin in the extracts of A. *muricata* could be responsible for the weight reduction in the rats. Saponin and tannin produces antinutritional effects that contributes to low absorption of nutrients and subsequently reduces weight. The result of the sub- acute toxicity on the liver of rat fed with AAM showed no anatomical changes, but has mild histological changes on the kidney of the rats after 28 days exposure to the extracts. The liver of rats revealed a regular tubular nucleoli and healthy vesicular nuclei which is similar to the liver of the rat fed with water while the kidney showed mild active interstitial congestion and inconspicuous tubular injury. Acutely non-toxic extracts may become toxic on long exposure which can be attributed to its accumulating effects on the enzyme level and consequent disruption of the anatomical structure (Gandhare *et al.*, 2013).

The limitation of the study are small sample sizes, lack of biochemical markers (ALT, AST and creatinine) tests to validate the histopathological studies, absence of mechanistic assays for the hypoglycaemic activity and lack of pharmacokinetic data. Hence, further studies should focus on the biochemical marker assays like >-glucosidase inhibition, insulin secretion, ALT, AST and creatinine assays for hypoglycaemic activity and toxicity studies, dose finding and mechanisms of action of the extracts as hypoglycaemic agent.

CONCLUSION

The acetone extract of *A. muricata* fruit peel exhibited hypoglycaemic activity comparable to glibenclamide at 1000 mg/kg in this study; however, mild renal histopathological changes after 28 days indicate the need for further safety studies.

Conflict of interest: The authors declare that there was no conflict of interest, and that the study was not sponsored.

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